PHYSICO-CHEMICAL STUDIES OF BIOCHEMICAL/BIOLOGICAL SYSTEMS

ABSTRACT

THESIS

Submitted for the Award of the Degree of

Doctor of Philosophy

IN

CHEMISTRY

BY

UZMA HASAN

DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

1999

Density, ultrasonic velocity and viscosity measurements have been made of

(i) ovalbumin- phosphate buffer (pH 2.4, 7.0 and 8.9) systems,

(ii) ovalbumin-maltose (1.5M)-phosphate buffer (pH 2.4, 7.0 and 8.9) systems and

(iii) amino acids (L-valine/L-serine)- urea (0.1M) – water systems

as functions of temperature and concentration of ovalbumin/amino acid in their respective systems.

The densities of the said systems as usual have been found to increase with increase in concentration and decrease with increase in temperature. The ultrasonic velocities increase with temperature and concentration. This may be attributed to the fact that the increase in temperature causes increase in the intermolecular distances resulting in an increase in the thermal motion of the molecules while an increase in concentration causes increase in the intermolecular interaction in the solutions.

From the density and ultrasonic velocity data, various derived parameters such as adiabatic compressibility (β_s), change in adiabatic compressibility ($\Delta\beta$) and its relative change (β_r), specific acoustic impedance (Z),Wada's constant (B) and molar sound velocity (R) have been evaluated. The adiabatic compressibility (β_s) in all the systems decreases with temperature as well as concentration. In case of ovalbumin–maltose buffer system, a marked decrease in the values of β_s (from those of system i) has been observed . This may be due to the presence of incompressible sugar molecules in the system (ii). The decrease in compressibility with temperature may be due to the thermal rupture of the solvation layer around the solute molecules. The plots of relative change in compressibility β_r as a function of concentration show that the intercepts are in the vicinity of zero in the systems (i) and (iii) indicating the presence of weak interaction due to very dilute nature of the systems. In case of systems (ii) , the intercepts are away from zero indicating the presence of strong interactions (or the strengthening of hydrophobic interaction) due to the presence of sugar maltose. The specific acoustic impedance

(Z) and the molar sound velocity (R) both increase with temperature as well as concentration of the solution. The plots of Z and R show a linear relationship of these parameters with concentration again indicating an almost ideal nature of the systems (i) and (iii) due to extremely dilute solutions. Wada's constant, B, also increases with temperature as well as concentration of the solutions .

The partial specific volumes ($\bar{v}^{\circ}$) and compressibilities ($\bar{\beta}_s$) of the systems (i) and (ii) were calculated from density and ultrasonic velocity. The values of partial specific volume and compressibility of compact native form of protein are 0.743 and 10.2139, respectively, at pH 7.0 and 298.15 K. The extremes of pH and temperature causes denaturation of proteins. This is evident from the higher values of $\bar{v}^{\circ}$ and $\bar{\beta}_s$ at pH 2.4 and 8.9. It is observed that the addition of maltose to the protein solutions decreases the values of $\bar{v}^{\circ}$ and $\bar{\beta}_s$ This may be attributed to the strengthening of hydrophobic interaction by maltose, resulting in the stabilisation of protein .

The density and ultrasonic velocity data have also been employed to calculate the other thermodynamic parameters such as isothermal compressibility (β_T), internal pressure (P_i), solubility parameter (δ), Pseudo-Grüneisen parameter (Γ) and surface tension (σ). The β_T values calculated from McGowan's and Pandey's relations agree well with each other. These values follow the trends as seen for β_s values. The values of β_T are slightly higher than those of β_s. The internal pressure (P_i) and the solubility parameter (δ) both increase with increase in temperature due to an increase in the repulsive forces among the molecules of the solution. The increase in the values of Γ with increase in temperature seems to be associated with an increase in the kinetic energy of the system. The surface tension of any liquid is the direct consequence of its cohesive forces. The variation of temperature and concentration effect these forces, therefore, surface tension decreases with increase in temperature and increase with increase in concentration.

The viscosity and its derived parameters provide information regarding the shapes and sizes of the molecules. Viscosity of all the systems increases with

concentration and decreases with increase in temperature. The values of intrinsic viscosity, [η] and shape factor, ν, calculated for the system (i), at pH 7.0 and 298.15 K are 3.0793 ml/g and 3.0026, respectively, indicating the globular form of protein. The increase in temperature and variation of pH denature the protein, therefore , the values of these parameters were increased. The stabilizing action of maltose is evident from the lowering of the values of [η] and ν in the systems (ii) when compared to those at pH 2.4 and 8.9 in the systems (i) without maltose. The viscosity data for amino acid–urea–water systems were analyzed in terms of Jones-Dole equation. The value of viscosity B-coefficient (always positive) does not provide any information about the structure-breaking or making ability of solute on solvent. The positive sign of dB/dT for L-serine shows its structure-breaking property on the solvent due to its polar side chain. The negative sign of dB/dT for L-valine shows its structure-making property due to its hydrophobic or non-polar side chain.

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Dedicated to my
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CERTIFICATE

This is to certify that the work presented in this thesis entitled ***"Physico-Chemical Studies of Biochemical/Biological Systems,"*** is original, carried out by ***Ms. Uzma Hasan*** under my supervision and is suitable for submission for the award of Ph.D. degree in Chemistry of this university.

(Prof. Bilquis N. Waris)

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Uzma Hasan

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CONTENTS

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GENERAL INTRODUCTION

Organisms tend to adapt their specific proteins to function efficiently within their normal environmental temperature [1-3]. This generally implies that proteins have a limited temperature range within which structural integrity is maintained. Outside this thermal span denaturation occurs with corresponding loss of function such as enzymatic activity. The thermal stability of a protein can be changed intrinsically by the addition of suitable stabilizing agents. It has been known for many years that sugars may protect proteins against loss of solubility during drying and may inhibit heat coagulation [4]. Simpson and Kauzmann [5] observed that the extent of denaturation of ovalbumin in urea solutions was reduced in the presence of sucrose. Gerlsma and Stuur [6] showed that the polyhydric alcohols raised the thermal transition temperatures of lysozyme and ribonuclease and Donovan [7] observed the stabilizing effect of sucrose on the proteins of egg white. Back et al [8] showed the increased thermal stability of proteins in the presence of sugars and polyols. Arakawa and Timasheff [9] observed the structural stabilization of proteins through their preferential interaction with the solvent components at high concentration of additives.

It is widely believed that the native conformation of a protein molecule in aqueous solution is chiefly stabilized by the hydrophobic interactions [10-12], i.e., the tendency of non-polar side chains to cluster in the interior of the protein and away from the surrounding water. These hydrophobic interactions are mainly effected by the sugars and polyhydric alcohols. This was shown by Back and his coworkes [8]. They measured the strength of hydrophobic interactions in model systems in sucrose and glycerol solutions. Inspite of a lot of information that has been obtained on the stability of proteins by different sugars, the volumetric, the compressibility and the viscometric behaviours of proteins in the presence of maltose sugar has not been studied so far. Therefore, in the present work we have tried to investigate the effect of maltose sugar on the stability of ovalbumin by studying the following systems:

(i). Ovalbumin – phosphate buffer system [pH 2.4, 7.0 and 8.9].

(ii). Ovalbumin – maltose – phosphate buffer system [pH 2.4, 7.0 and 8.9].

For this purpose, the density, the ultrasonic velocity and the viscosity of the above systems have been measured under varying conditions of concentration and temperature.

In recent years, mixed aqueous solvents have been used extensively by many workers [13-20] in different fields to control the factors such as solubility, reactivity and stability of the systems. Since urea is highly soluble in water, urea-water mixtures have been used as solvents for extensive experimental investigations [13,14,16,20]. Urea is an important biomolecule. It is a non-electrolyte and hydrophillic structure-breaker. It is generally accepted that the addition of urea to water increases the hydrogen bonding in solution. Urea does not interact with either hydrophillic or hydrophobic groups or molecules but takes active part in the hydrogen bonding among water molecules in aqueous medium [21]. So the urea solution is similar to water but with less structure. But a molecular dynamics calculation [22] has shown that a urea molecule can enter into the water structure without breaking it noticeably. Consequently, urea can't be easily classified into a net structure-maker or a structure-breaker. Urea is also known to cause denaturation in proteins. It changes the native conformation of proteins to the denatured random coil one. On the other hand,the thermodynamic properties of mixing suggest that the interactions between the urea and the water molecules in urea-water solutions are similar to those of water-water interaction in pure water [23]. Therefore, urea-water solutions are often treated as ideal solutions for extensive experimental investigations.

Water constitutes upto 70 percent or more of the weight of most forms of life. Because water pervades all portions of every cell, it is the medium in which the transport of nutrients, enzyme-catalysed reactions of metabolism and the transfer of chemical energy occurs. Therefore, all aspects of cell structure and functions are necessarily adapted to the physical and chemical properties of water and this is the reason behind the increasing interest to study the states of water in the living cells. For this purpose, it will be necessary to study the physico-chemical properties of the simple model systems under various conditions. Amino acids are

the most convenient, low molecular weight substances representing the simple models. These are the basic constituents of a number of compounds of biological relevance such as proteins, hormones, anti-biotics, enzymes etc. Due to the structural complexities it is extremely difficult to carry out experimental thermodynamic studies on these macromolecules. In such cases amino acids serve as the useful model compounds. In the present work, the densities, the ultrasonic velocities and the viscosities were measured for amino acids (L-valine and L-serine)-urea-water ternary systems at various concentrations and temperatures. From these measurements various derived parameters of density, ultrasonic velocity and viscosity were evaluated.

The ultrasonic velocity in solutions has been proved to be a significant physical property that provides useful informations regarding the nature and the extent of intermolecular/interionic interaction occurring in solutions. Different workers have proposed different theories for the calculation of ultrasonic velocity [24-34]. Various attempts have been made on the calculation of ultrasonic velocity in pure organic liquids [35,36], their binary [37-40], ternary [41-46] and quaternary mixtures [47]. The aqueous solutions of electrolytes [48-50] and non-electrolytes [51-52] have also been studied. Ultrasonic velocity of biological macromolecules like amino acids [53-59] and proteins [60-64] have also been measured experimentally in aqueous [53,56-61,65-67] as well as mixed aqueous [20,64,68] solvents. Such data alongwith the density data have been employed for the calculation of the derived parameters like adiabatic compressibility, β_s, compressibility lowering, $\Delta\beta$, specific acoustic impedance, Z, etc. These parameters provide information about the physical nature of aggregates occurring in solutions [69].

Using the density and the sound velocity in protein solutions the partial specific volume and compressibility were evaluated. The partial specific volume of a protein is a characteristic parameter that has been used to elucidate several processes that depend upon the protein conformation or during which the protein conformation changes. Since the early volumetric studies of small organic

compounds by Traube [70], there have been numerous investigations [53,59-67,71-75] on the partial specific volume and compressibility of amino acids and proteins, since the accurate measurements of sound velocity became possible in dilute solutions. An important result obtained from such studies was that the globular proteins have positive compressibility indicating the great contribution of the internal cavity in the structure of protein.

Another derived parameter, the isothermal compressibility, has been the subject of interest for a number of workers. Various equations [76-79] have been given to evaluate the isothermal compressibility for various systems [80,81]. In the present work, it has been calculated using the empirical relation given by McGowan in 1966 [79]. An alternative expression given by Pandey et al [82], for the evaluation of isothermal compressibility, was also tested. It was found that the results obtained from both were in good agreement with each other.

Internal pressure, a fundamental property of the liquid state, has been studied initially by Hildebrand and Scott [83-84] and subsequently by several other workers [85-91]. It has been extensively used to investigate the molecular interactions in binary liquid mixtures [88,89,92-94]. Hildebrand and Soctt introduced a parameter known as solubility parameter in the theory of solution. The importance of this parameter has been demonstrated by a number of workers [95,96]

The Pseudo-Grüneisen parameter, a dimensionless constant, is governed by the molecular order and structure. It had been the subject of study for solids [97,98] and was later extended for liquids as well [99]. Later the utility of this parameter was extended to the structural study of liquids by defining its Pseudo counterpart [100]. This parameter is related to the thermal expansion coefficient and the specific heat ratio.

The surface tension is an important phenomenon in the study of molecular chemistry. It is the direct consequence of the cohesive forces between the

molecules of a liquid. Hence, it is an important physical property, which has been studied to get the information about the intermolecular interactions in solutions.

The transport properties in solution are studied by measuring the viscosity of the solutions. The viscosity measurement of macromolecules provides information regarding the shape and size of these molecules [101]. Several theories have been given to evaluate the viscosity of binary liquid mixtures [102-108]. These theoretical relations have been used to explain the strength and the nature of interactions in these systems [104,105]. Several workers have carried out the experimental viscometric measurements in aqueous as well as mixed aqueous solutions of biological macromolecules to evaluate some thermodynamic parameters such as association constant, change in free energy of activation and enthalpy change etc [109-117]. The evaluation of intrinsic viscosity of protein under varying conditions of temperature, pH and the addition of cosolvents help in detecting the conformational changes in protein. The extremes of pH and temperature cause the loss of biological activity and the protein is said to be denatured. The addition of cosolvents like sugars increases the stability of protein and protect it against thermal and pH denaturation. For the native conformation of protein, the value of intrinsic viscosity lies between 3-4 ml/g and for the denatured states, its value goes beyond 4 ml/g.

The viscosity data have also been interpreted by several workers in terms of Jones-Dole equation [19,118,-123]. They have introduced the viscosity coefficient B for the dipolar ions, particularly amino acids. It is argued that the sign of the temperature dependence of the B-coefficient provides a more satisfactory information about the structure-making or structure-breaking ability of the solutes on the solvent than the sign of the B-coefficient.

In order to get a complete picture of the physical nature of solutes in solution and the type and the strength of interactions between them we have measured the density, ultrasonic velocity and viscosity of the said systems. From these measurements different derived parameters were evaluated.

EXPERIMENTAL

MATERIAL AND SAMPLE PREPARATION

Crystallized and lyophilized ovalbumin from Sigma Chemical Co. (LOT 106 H 7070, Grade V) was used for sample preparation. 0.2 molar aqueous solutions of both monobasic and dibasic sodium phosphate (purchased from E. Merck) were mixed in different proportions to prepare phosphate buffers of pH 2.4, 7.0 and 8.9. The pH of these solutions were measured by digital pH meter (Elico Pvt. Ltd. Hyderabad, model T-10).For the first half of the experiment, these phosphate buffers were used as solvents for preparing four solutions of different protein concentrations (4.0-10.0 x 10^{-3} g/ml). For the next half of the experiment, 1.5 molar solution of maltose (SD fine chemicals, India) prepared in phosphate buffers(pH 2.4, 7.0 and 8.9) were used as solvents. The protein concentrations remained unchanged.

The amino acids L-valine and L-serine (SRL Mumbai, India) were extrapure and used without further purification. The amino acids were dried before sample preparation. 0.1 molar aqueous urea solution was taken as solvent for the preparation of amino acid solutions. Urea was purchased from Qualigens Co. India. Triply distilled water was used for preparing aqueous solutions. The resulting solutions were

i. Ovalbumin in phosphate buffers (pH 2.4, 7.0 and 8.9).
ii. Ovalbumin in maltose + phosphate buffers (pH 2.4, 7.0 and 8.9) mixtures.
iii. L-valine in urea-water mixture.
iv. L-serine in urea-water mixture.

TEMPERATURE CONTROL

For the measurement of density and viscosity, a thermostated paraffin bath was used to maintain a uniformity in temperature throughout the course of experiment. The paraffin bath of about 5 litres capacity consisted of an immersion heater of 1500 Watts, a Remi stirrer, a check thermometer of least count 0.1 ^{0}C, a contact thermometer [TGL 4850 NAV = 0.03A, Un= 250V (GDR)] and a relay

[Jumo, NT 10.0, 220V ≅ 6 A (W. Germany)] to control the variation in temperature. The variation in temperature was about $\pm 0.1\ ^{0}C$.

The ultrasonic interferometer was used to measure the ultrasonic velocity of the samples in the temperature range 25.0^{0} to $50.0\ ^{0}C$. Water from an ultrathermostat was allowed to circulate through the insulated double walled jacket of the cell. The thermal stability was found to be $\pm 0.1\ ^{0}C$.

DENSITY MEASUREMENT

A pyknometer, consisted of a small bulb with a flat bottom (of about 5 ml capacity) and a graduated stem, was used to measure the density of the experimental liquid. The pyknometer was calibrated with triply distilled water. The clean and dried pyknometer was weighed and filled with triply distilled water and again weighed. The mass of the distilled water was determined by the difference in these two masses. Then the pyknometer was immersed in the paraffin bath maintained at the required temperature and the changes in temperature corresponding to the changes in volume at each mark were recorded. The density of pure water at these temperatures corresponding to each mark was obtained from a standard relation:

$$\rho = 1.000\ 525 - 2 \times 10^{-5} t - 4.72 \times 10^{-6} t^{2}$$

where t is the temperature in ^{0}C. From the known values of mass and density of water, the volume corresponding to each mark of the pyknometer was determined.

This experiment was repeated with different masses of water. Using the known values of mass and volumes, the densities at the required temperatures were determined. The values of the observed densities were compared with those of the reported ones. It was found that the accuracy of the measurement was within $\pm\ 0.0002\ g\ cm^{-3}$.The densities of the test solutions were determined by using the calibrated pyknometer.

VISCOSITY MEASUREMENT

The viscosity measurement has been made with a Cannon-Ubbelohde viscometer. The viscometer consists of three arms: receiving, measuring and auxiliary for forming the suspended level arrangement. All the three arms are parallel to each other. The receiving and the measuring arms form a U through a bulb D. The measuring arm has two bulbs A and B. On the upper and lower side of the bulb, the two marks were used to record the time of fall of the solution. The auxiliary arm was sealed to the receiving arm through a bulb C. In between the bulbs B and C, there is a fine capillary of suitable dimensions. The viscometer has been designed in such a way that the center of gravity of the three bulbs A,B and C was aligned vertically. This reduces the acceleration due to gravity and thus minimizes the experimental errors. The surface tension correction of the viscometer was negligible and the transport of momentum was carried out freely under the weight of the total volume of the test liquid. This was due to a special feature of this type of viscometer that the capillary effects of the two liquid surfaces are neutralized by each other.

The calibration was done by using triply distilled water. The viscometer was filled with triply distilled water whose amount was sufficient to avoid the entrance of any air bubble into the capillary while fudicial bulb B was filled. The viscometer was clamped in the vertical position in the thermostated paraffin bath for about half an hour before recording the time of fall to avoid thermal fluctuation in the viscometer. Then the sample was sucked into the bulb A where it was allowed to stand for some time. Then the liquid was allowed to fall and the time of fall was recorded. This process was repeated several times and the similar readings were taken at each required temperature. The time of fall was recorded with a stop watch of accuracy ± 0.1 second. Poiseuille's equation

$$\eta = \pi \rho h g r^4 t / 8LV = \rho \beta t$$

was employed to calculate the viscosities using the density and the time of fall of the solutions. In the above equation, ρ is the density of the solution, h is the height of the column in the viscometer, g is the acceleration due to gravity, r is the radius of the capillary, L is the length and t is the time of fall of the test liquid of volume V. The terms associated with a given viscometer have been denoted by single term β , which is a constant for a particular viscometer. β has been calculated by using the reported values of viscosities of water at several temperatures. The accuracy of the calibrated viscometer was checked by measuring the viscosities of water at test temperature and then comparing the experimental values with the reported ones. The accuracy was found to be $\pm 0.2\%$.

MEASUREMENT OF ULTRASONIC VELOCITY

The measurement of ultrasonic velocity has been done by ultrasonic interferometer. It is a simple and direct device to determine the ultrasonic velocity in liquids with high degree of accuracy. The ultrasonic interferometer (Mittal's Model F-81) of a single frequency of 4 MHz was used for the measurement of sound velocity in the experimental temperature range.

The working principle of the instrument is based on the accurate determination of the wavelength (λ) in the medium. The ultrasonic waves of known frequency, ν, are produced by a quartz plate fixed at the bottom of the cell. These are reflected back to the quartz plate by a movable metallic plate kept parallel to the quartz plate. If the separation between these two plates is exactly a whole multiple of sound wavelength, standing waves are produced in the medium. This acoustic resonance gives rise to an electrical reaction on the generator driving the quartz plate and the anode current of the generator becomes maximum.

If the distance between the two plates is increased or decreased and the separation is exactly one-half of the wavelength or multiple of it, then again the anode current becomes maximum. Knowing the values of wavelength, the ultrasonic velocity in the medium can be obtained from the equation

$$U = \lambda \times \nu$$

The ultrasonic interferometer consists of the following two parts:

i. The High Frequency Generator.

ii. The Measuring Cell.

High Frequency Generator : It is designed to excite the quartz plate at its resonance frequency to generate ultrasonic waves in the liquid filled in the measuring cell. A microammeter is provided on the high frequency generator to observe the changes in current. There are two controls, one for the purpose of sensitivity regulation and the other for the initial adjustment of microammeter.

Measuring Cell : It is a specially designed double-walled cell for maintaining the temperature of the liquid constant during the experiment. On the top of it is a fine micrometer screw which can lower or raise the reflector plate in the cell through a known distance. It has a quartz plate fixed at its bottom.

The instrument was adjusted in the following way:

1. The cell was inserted in the square-base socket and clamped to it by a screw provided on one of its sides.

2. The curled cap of the cell was unscrewed and removed. Then the test solution was filled in it and the cap was screwed.

3. Water was circulated through the two tubes in the double-walled cell in order to maintain the desired temperature during the experiment.

4. The cell was connected with a high frequency generator by a co-axial cable provided with the instrument.

5. The generator was given 15 seconds warming up time before recording readings.

6. The sudden rise or fall in temperature of the circulated liquid was avoided to prevent the thermal shock to the quartz crystal.

Two knobs are provided on the high frequency generator, one is marked with 'Adj' and the other with 'Gain'. The knob marked with 'Adj' was used to adjust the position of the needle on the ammeter and the knob marked with 'Gain' was used to increase the sensitivity of the instrument for greater deflection. The microammeter was used to record the maximum deflections by adjusting the micrometer screw.

The measuring cell was connected to the output terminal of the high frequency generator by a shielded co-axial cable. Before switching on the generator, the measuring cell was fixed to its base and filled with the experimental liquid. The ultrasonic waves produced by the excited quartz-crystal move normal from the crystal till they are reflected back from the movable plate and the standing waves are formed in the liquid in between the reflector (movable) plate and the quartz crystal.

The micrometer screw was slowly raised to record the maximum anode current. The wavelength was determined by recording the total distance moved by the micrometer for twenty maxima of anode current. The distance (d) thus moved by the micrometer gives the value of wavelength (λ) by using the relation

$$d = n \times \lambda/2$$

where n is the number of maxima in anode current. Once the wavelength (λ) is known, the velocity (U) in the liquid can be calculated with the help of the relation.

$$U = \nu \times \lambda/2$$

The accuracy in the measurement was found to be within $\pm 0.07\%$.

CHAPTER -1

THE INTERMOLECULAR INTERACTIONS AS REFLECTED BY ULTRASONIC VELOCITY

INTRODUCTION

The ultrasonic velocity in liquids has been known to be a significant physical property that either directly or through its derived parameters provides a basis for understanding the nature of intermolecular or interionic interactions occurring in solutions.

During recent past considerable interest has been developed in the ultrasonic studies of liquids, their mixtures, the aqueous solutions of electrolytes and many compounds of biological importance. Experimental data of ultrasonic velocity were analysed theoretically in the light of various empirical theories. Studies on the ultrasonic velocity and the compressibility of aqueous solutions of proteins have started a long time ago [124, 125] and till now much work has been done on the compressibility of amino acids and proteins in aqueous solutions. Mixed aqueous solvents have also been used for these measurements. Such data have been used for the calculation of some very useful thermodynamic properties. In the present work, an attempt has been made to calculate the derived parameters such as adiabatic compressibility, β_s , compressibility lowering, $\Delta\beta$, specific acoustic impedance, Z, Wada's constant, B and molar sound velocity, R using the ultrasonic velocity and density of the following systems :

(i) ovalbumin – phosphate buffer system [pH 2.4, 7.0 and 8.9],
(ii) ovalbumin – maltose – phosphate buffer system [pH 2.4, 7.0 and 8.9],
(iii) aminoacid (L-valine/L-serine)-urea-water system,
over a wide range of temperature and concentration of the solutes.

THEORY

Adiabatic compressibility, β_s , has been calculated using the Laplace equation:

$$\beta_s = U^{-2} \rho^{-1} \qquad 1.1$$

where U is the ultrasonic velocity and ρ is the density of the solution. The compressibility lowering, $\Delta\beta$, is calculated from the difference between the compressibilities of the solvent, β^0 and the solution β_s. Thus

$$\Delta\beta = \beta^0 - \beta_s \qquad 1.2$$

Relative change in compressibility is given by

$$\beta_r = \Delta\beta / \beta^0 \qquad 1.3$$

The specific acoustic impedance, Z, the molar sound velocity, R and the Wada's constant, B, are defined by the following relations:

$$Z = U.\rho \qquad 1.4$$

$$* \qquad R = (M/\rho)\,U^{1/3} = V_m\ U^{1/3} \qquad 1.5$$

$$* \qquad B = V_m\ \beta_s^{-1/7} \qquad 1.6$$

where V_m is the molar volume and M is the molecular weight of the solution given by

$$M = M_1 X_1 + M_2 X_2 + M_3 X_3 \qquad 1.7$$

where M_1, M_2 and M_3 are the masses of the different species of the solution and X_1, X_2 and X_3 are their mole fractions.

* R and B have been calculated only for amino acid-urea-water systems.

RESULTS AND DISCUSSION

The densities and the ultrasonic velocities of the systems under investigation have been least-squares fitted to the equations 1.8 and 1.9, respectively,

$$\rho = \Sigma^2_{i=0}\ \rho_i t^i \qquad 1.8$$

$$U = \Sigma^2_{i=0}\ U_i t^i \qquad 1.9$$

Table 1.1: Least-squares fitted parameters of the density equation (1.8) as a function of concentration for the following systems:

(a) Ovalbumin- Buffer System (pH 2.4)

Concentration g/ml	ρ_0	ρ_1	$\rho_2 x10^3$	Standard Deviation
0.000	158.974	5.82511	−10.0002	0.0365
0.004	270.727	5.11261	−08.8571	0.0390
0.006	160.207	5.82489	−09.9998	0.0365
0.008	161.824	5.82100	−10.0000	0.0365
0.010	080.419	6.35113	−10.8569	0.0138

(b) Ovalbumin-Maltose-Buffer System (pH 2.4)

Concentration g/ml	ρ_0	ρ_1	$\rho_2 x10^3$	Standard Deviation
0.000	−888.636	13.99470	−23.42810	$1.950x10^{-2}$
0.004	376.176	05.75300	−10.00000	$3.149x10^{-5}$
0.006	376.511	05.75278	−09.99965	$3.231x10^{-5}$
0.008	510.523	04.87900	−08.57213	$1.950x10^{-2}$
0.010	482.057	05.06083	−08.85742	$3.910x10^{-2}$

(c) Ovalbumin- Buffer System (pH 7.0)

Concentration g/ml	ρ_0	ρ_1	$\rho_2 x10^3$	Standard Deviation
0.000	432.063	4.07203	−7.14268	0.0138
0.004	436.677	4.05993	−7.14250	0.0390
0.006	242.814	5.30676	−9.14289	0.0390
0.008	486.602	3.72374	−6.57104	0.0195
0.010	620.782	2.84952	−5.14282	0.0138

(d) ***Ovalbumin-Maltose-Buffer System (pH 7.0)***

Concentration g/ml	ρ_0	ρ_1	$\rho_2 \times 10^3$	Standard Deviation
0.000	0902.745	2.36133	−4.57136	1.950×10^{-2}
0.004	0824.872	2.87989	−5.42899	1.950×10^{-2}
0.006	0959.051	2.00502	−3.99972	1.965×10^{-5}
0.008	1041.690	1.47469	−3.14244	3.900×10^{-2}
0.010	1015.520	1.64894	−3.42843	1.950×10^{-2}

(e) ***Ovalbumin-Buffer System (pH 8.9)***

Concentration g/ml	ρ_0	ρ_1	$\rho_2 \times 10^3$	Standard Deviation
0.000	570.979	3.18961	−5.71411	0.0458
0.004	266.400	5.14883	−8.85742	0.0976
0.006	−166.921	7.96583	−13.4283	0.0414
0.008	242.189	5.31855	−9.14254	0.0391
0.010	214.817	5.49702	−9.42889	0.0195

(f) ***Ovalbumin-Maltose-Buffer System (pH 8.9)***

Concentration g/ml	ρ_0	ρ_1	$\rho_2 \times 10^3$	Standard Deviation
0.000	425.086	5.41692	−9.42871	0.0196
0.004	507.524	4.88657	−8.57143	0.0195
0.005	452.587	5.24308	−9.14342	0.0390
0.008	481.991	5.06061	−8.85707	0.0390
0.010	594.532	4.34402	−7.71380	0.0276

(g) ***L-Valine-Urea-Water System***

Concentration mol/kg	ρ_0	ρ_1	$\rho_2 \times 10^3$	Standard Deviation
0.0000	812.144	1.56312	−3.14314	0.0391
0.0502	816.653	1.55901	−3.14296	0.0390
0.1008	762.449	1.91531	−3.71460	0.0276
0.1520	786.551	1.75283	−3.42826	0.0414
0.2035	818.189	1.56701	−3.14296	0.0138
0.2555	794.454	1.73727	−3.42896	0.0195
0.3081	795.904	1.73694	−3.42843	0.0195
0.3610	825.153	1.55901	−3.14296	0.0390
0.4144	801.453	1.72905	−3.42861	0.0195

(h) ***L-Serine-Urea-Water System***

Concentration mol/kg	ρ_0	ρ_1	$\rho_2 \times 10^3$	Standard Deviation
0.1001	195.505	5.52492	−9.42871	1.950×10^{-2}
0.2002	323.224	4.77242	−8.28561	2.760×10^{-2}
0.3004	356.423	4.59038	−7.99997	2.462×10^{-5}
0.4005	357.957	4.59838	−7.99997	3.650×10^{-2}
0.5006	305.321	4.95868	−8.57160	1.950×10^{-2}
0.6007	370.939	4.58270	−8.00049	3.650×10^{-2}
0.7009	287.836	5.12862	−8.85707	1.380×10^{-2}
0.8010	435.542	4.22197	−7.42815	1.950×10^{-2}
0.9014	271.596	5.29487	−9.14307	4.360×10^{-2}
1.0012	190.891	5.83322	−10.00030	3.484×10^{-5}

Table 1.2: Experimental values of density (kg m^{-3}) as functions of temperature and concentration for the following systems:

(a) Ovalbumin-Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	1006.8	1005.8	1004.4	1002.5	1000.0
0.004	1007.7	1006.7	1005.1	1003.2	1000.8
0.006	1008.0	1007.0	1005.6	1003.7	1001.2
0.008	1008.4	1007.5	1006.0	1004.0	1001.6
0.010	1008.9	1008.0	1006.6	1004.6	1002.1

(b) Ovalbumin-Maltose-Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	1202.0	1200.8	1199.0	1196.4	1193.7
0.004	1202.5	1201.2	1199.4	1197.1	1194.3
0.006	1202.8	1201.5	1199.7	1197.4	1194.6
0.008	1203.2	1201.8	1200.0	1197.8	1195.1
0.010	1203.6	1202.2	1200.5	1198.3	1195.6

(c) Ovalbumin-Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	1011.2	1010.1	1008.6	1006.8	1004.6
0.004	1012.2	1011.1	1009.5	1007.6	1005.4
0.006	1012.3	1011.3	1009.9	1008.1	1005.9
0.008	1012.7	1011.6	1010.1	1008.3	1006.2
0.010	1013.2	1012.0	1010.5	1008.8	1006.8

(d) ***Ovalbumin-Maltose-Buffer System (pH 7.0)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	1200.4	1198.5	1196.3	1193.9	1191.3
0.004	1200.9	1199.0	1196.8	1194.3	1191.7
0.006	1201.3	1199.3	1197.1	1194.7	1192.1
0.008	1202.0	1200.0	1197.7	1195.3	1192.8
0.010	1202.4	1200.3	1198.1	1195.7	1193.1

(e) ***Ovalbumin-Buffer System (pH 8.9)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	1014.0	1012.8	1011.3	1009.4	1007.4
0.004	1014.1	1013.4	1011.9	1010.1	1008.0
0.006	1014.4	1013.9	1012.6	1010.8	1008.2
0.008	1015.2	1014.3	1013.0	1011.1	1008.9
0.010	1015.6	1014.7	1013.4	1011.6	1009.3

(f) ***Ovalbumin-Maltose-Buffer System (pH 8.9)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	1202.0	1200.7	1199.0	1196.8	1194.1
0.004	1202.5	1201.2	1199.4	1197.2	1194.6
0.006	1203.0	1201.8	1200.0	1197.8	1195.2
0.008	1203.5	1202.1	1200.4	1198.2	1195.5
0.010	1204.0	1202.5	1200.7	1198.4	1195.8

(g) ***L-Valine-Urea-Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.0000	0998.8	0997.1	0995.4	0993.4	0991.3
0.0502	1002.1	1000.4	0998.6	0996.7	0994.5
0.1008	1003.3	1001.7	0999.9	0998.0	0995.8
0.1520	1004.4	1002.9	1001.1	0999.3	0997.2
0.2035	1006.0	1004.4	1002.6	1000.7	0998.6
0.2555	1007.6	1006.0	1004.2	1002.2	1000.1
0.3081	1009.0	1007.4	1005.6	1003.6	1001.5
0.3610	1010.6	1008.9	1007.1	1005.2	1003.0
0.4140	1012.2	1010.5	1008.7	1006.7	1004.5

(h) ***L-Serine-Urea-Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.1001	1004.6	1003.9	1002.7	1001.0	0998.9
0.2002	1009.6	1008.5	1007.1	1005.2	1002.9
0.3004	1013.9	1012.8	1011.3	1009.4	1007.1
0.4005	1017.8	1016.8	1015.3	1013.4	1011.2
0.5006	1021.8	1020.8	1019.4	1017.6	1015.3
0.6007	1026.1	1024.9	1023.4	1021.5	1019.1
0.7009	1029.6	1028.6	1027.2	1025.3	1023.0
0.8010	1034.0	1032.8	1031.2	1029.2	1026.9
0.9014	1037.5	1036.5	1035.0	1033.1	1030.7
1.0012	1041.1	1040.2	1038.8	1036.9	1034.5

Table 1.3: Least-squares fitted parameters of the ultrasonic velocity equation 1.9 as a function of concentration for the following systems:

(a) Ovalbumin- Buffer System (pH 2.4)

Concentration g/ml	U_0	U_1	$U_2 x 10^3$	Standard Deviation
0.000	−069.3799	8.70321	−11.42820	0.4930
0.004	069.0360	7.81700	−10.00000	0.3760
0.006	170.8390	7.13683	−08.85742	0.3289
0.008	249.8390	6.61848	−08.00014	0.2422
0.010	−153.0210	9.24977	−12.28900	0.3959

(b) Ovalbumin-Maltose-Buffer System (pH 2.4)

Concentration g/ml	U_0	U_1	$U_2 x 10^3$	Standard Deviation
0.000	701.079	5.81653	−8.28578	0.1242
0.004	790.392	5.26597	−7.42815	0.0552
0.006	655.209	6.14819	−8.85638	0.0828
0.008	632.049	6.31509	−9.14342	0.0138
0.010	743.958	5.60249	−8.00014	0.0365

(c) Ovalbumin-Buffer System (pH 7.0)

Concentration g/ml	U_0	U_1	$U_2 x 10^3$	Standard Deviation
0.000	067.4623	7.85300	−1.00000	0.3651
0.004	−037.5511	8.54917	−1.11426	0.4019
0.006	068.9623	7.85300	−1.00000	0.3651
0.008	125.0140	7.49692	−0.94287	0.3231
0.010	−098.5439	8.92969	−1.17146	0.3475

(d) ***Ovalbumin-Maltose-Buffer System*** ***(pH 7.0)***

Concentration g/ml	U_0	U_1	$U_2 \times 10^3$	Standard Deviation
0.000	0854.130	4.86232	−6.85756	0.0744
0.004	0939.965	4.32376	−5.99993	0.0731
0.006	1132.750	3.08503	−3.99972	0.1826
0.008	1163.710	2.89920	−3.71443	0.1952
0.010	1251.630	2.35264	−2.85679	0.2313

(e) ***Ovalbumin-Buffer System*** ***(pH 8.9)***

Concentration g/ml	U_0	U_1	$U_2 \times 10^3$	Standard Deviation
0.000	−212.659	9.74811	−1.31431	0.2855
0.004	−235.787	9.91416	−1.34288	0.2537
0.006	−146.880	9.36403	−1.25718	0.2973
0.008	069.576	7.95883	−1.02853	0.3662
0.010	071.443	7.95526	−1.02860	0.4755

(f) ***Ovalbumin-Maltose-Buffer System*** ***(pH 8.9)***

Concentration g/ml	U_0	U_1	$U_2 \times 10^3$	Standard Deviation
0.000	0990.683	3.99546	−5.42829	0.0552
0.004	0941.476	4.33597	−6.00028	0.0730
0.006	0999.180	3.97589	−5.42899	0.0552
0.008	1087.350	3.42954	−4.57171	0.0552
0.010	0926.998	4.48223	−6.28627	0.0586

(g) ***L-Valine-Urea-Water System***

Concentration mol/kg	U_0	U_1	$U_2 x 10^3$	Standard Deviation
0.0000	-0527.901	11.58120	-16.0006	0.1713
0.0502	0460.473	05.21393	-05.7146	0.3857
0.1008	1027.030	01.62800	-00.0000	0.4844
0.1520	0949.657	02.24439	-01.1429	0.4749
0.2035	1600.850	-01.96550	05.7139	0.5780
0.2555	1845.800	-03.53653	08.2857	0.6435
0.3081	0755.783	03.62516	-03.4287	0.4930
0.3610	-0239.978	10.13020	-14.0001	0.3142
0.4144	-1614.340	19.09660	-28.5714	0.1171

(h) ***L-Serine-Urea-Water System***

Concentration mol/kg	U_0	U_1	$U_2 x 10^3$	Standard Deviation
0.1001	-919.202	14.01170	-19.7140	0.4204
0.2002	-359.616	10.63050	-14.5717	0.0915
0.3004	186.257	07.29291	-09.4287	0.1518
0.4005	391.745	06.11644	-07.7144	0.3358
0.5006	530.501	05.33184	-06.5712	0.4325
0.6007	519.255	05.47410	-06.8572	0.5101
0.7009	411.086	06.19427	-07.9998	0.4844
0.8010	072.173	08.36764	-11.4289	0.3937
0.9014	-361.201	11.11090	-15.7146	0.1656
1.0012	-890.278	14.42830	-20.8573	0.0743

where t is the temperature in Kelvin. The coefficients of the density and the ultrasonic velocity are given in tables 1.1 and 1.3 along with the standard deviations.

The ultrasonic velocities for different systems are plotted against concentration at various temperatures [Fig. 1.1 a–h]. The plots show the increase in the values of ultrasonic velocity with the corresponding increase in temperature as well as the concentrations of amino acid/protein in their respective systems. This increase may be attributed to an increase in the intermolecular interactions with the increase in temperature and concentration.

The adiabatic compressibility (Table 1.5), obtained from the measurement of sound velocity, is determined primarily by intermolecular and interionic interactions. As seen from the plots [Figs 1.2 a-h], the compressibility decreases from pure water to the solutions and it is found to decrease with the increase in the concentration of the solutes as well as the temperature [17,18,51] .

It is assumed that liquid water is an equilibrium mixture of two classes of molecules [126]. Class I is constituted by hydrogen–bonded molecules (open or ice-like structure) and class II is constituted by unbonded monomers (close-packed structure). This structure was supported by several workers [127-129]. The dimer, trimer, tetrarmer, pentamer as well as hexamer structure for liquid water have been established so far. In all these structures water is tetrahedrally-coordinated [130]. The hexagonally arranged water molecules form quite bulky structure with large empty spaces within the framework of molecules. These spaces accommodate the unbonded monomeric water molecules or other non-charged small molecules. In addition to the above-mentioned polymeric forms, Dang [131] reported the additional molecular dynamic's results indicating the presence of water octamers, nanomers and decamers. The lowest minimum-energy structures for water nanomers and decamers have not yet been established.

Table 1.4: Experimental values of ultrasonic velocities (U, ms^{-1}) as functions of temperature and concentration for the following systems:

(a) Ovalbumin- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	1509.8	1518.4	1527.1	1536.0	1542.5
0.004	1510.9	1519.5	1528.1	1536.8	1544.0
0.006	1511.5	1520.0	1529.0	1537.5	1544.7
0.008	1512.1	1520.8	1529.6	1538.2	1545.6
0.010	1512.8	1521.8	1530.4	1539.3	1546.0

(b) Ovalbumin-Maltose-Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	1698.7	1703.0	1706.5	1710.1	1712.9
0.004	1700.1	1704.2	1707.7	1711.0	1713.9
0.006	1701.0	1705.2	1708.7	1712.1	1714.8
0.008	1702.1	1706.2	1709.8	1713.0	1715.7
0.010	1703.2	1707.1	1710.7	1713.9	1716.6

(c) Ovalbumin- Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	1520.0	1529.0	1537.5	1546.5	1553.5
0.004	1521.0	1530.0	1538.5	1547.5	1554.3
0.006	1521.5	1530.5	1539.0	1548.0	1555.0
0.008	1522.2	1531.0	1539.7	1548.5	1555.6
0.010	1522.6	1531.8	1540.5	1549.5	1556.5

(d) ***Ovalbumin-Maltose-Buffer System (pH 7.0)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	1694.2	1698.0	1701.3	1704.2	1707.0
0.004	1695.7	1699.4	1702.6	1705.5	1708.3
0.006	1696.9	1700.6	1703.6	1706.4	1709.5
0.008	1697.8	1701.5	1704.3	1707.2	1710.2
0.010	1699.0	1702.6	1705.1	1708.2	1711.0

(e) ***Ovalbumin- Buffer System (pH 8.9)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	1525.5	1534.5	1543.0	1551.5	1558.2
0.004	1526.5	1535.4	1544.0	1552.3	1559.0
0.006	1527.6	1536.2	1544.8	1553.0	1559.6
0.008	1528.4	1536.7	1545.4	1553.7	1560.4
0.010	1529.2	1537.3	1546.1	1554.5	1561.0

(f) ***Ovalbumin-Maltose-Buffer System (pH 8.9)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	1699.4	1703.0	1706.5	1709.5	1712.4
0.004	1700.9	1704.4	1707.9	1710.9	1713.6
0.006	1702.0	1705.5	1708.9	1711.8	1714.6
0.008	1703.5	1706.8	1710.1	1713.0	1715.7
0.010	1704.6	1708.0	1711.3	1714.2	1716.7

(g) ***L-Valine-Urea-Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.0000	1502.7	1512.5	1521.3	1529.9	1537.0
0.0502	1507.2	1515.4	1524.9	1532.8	1540.8
0.1008	1512.7	1519.9	1529.0	1537.1	1544.8
0.1520	1517.5	1524.4	1532.9	1540.8	1547.8
0.2035	1523.1	1529.4	1537.9	1546.2	1553.6
0.2555	1528.3	1534.4	1542.9	1551.5	1559.0
0.3081	1532.1	1539.1	1547.3	1555.3	1561.8
0.3610	1536.0	1544.1	1552.2	1559.8	1565.7
0.4144	1539.5	1549.1	1557.1	1564.1	1569.2

(h) ***L-Serine-Urea-Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.1001	1506.2	1516.2	1526.7	1535.7	1543.0
0.2002	1514.6	1523.8	1532.5	1540.5	1547.5
0.3004	1522.4	1530.8	1538.2	1545.3	1552.2
0.4005	1529.4	1547.4	1543.9	1550.3	1557.0
0.5006	1535.8	1543.5	1549.4	1555.4	1561.9
0.6007	1541.5	1549.2	1554.8	1560.6	1567.0
0.7009	1546.5	1554.3	1560.1	1565.9	1572.3
0.8010	1550.8	1559.0	1565.3	1571.4	1577.7
0.9014	1554.5	1563.1	1570.4	1577.0	1583.2
1.0012	1557.5	1566.8	1575.3	1582.7	1588.9

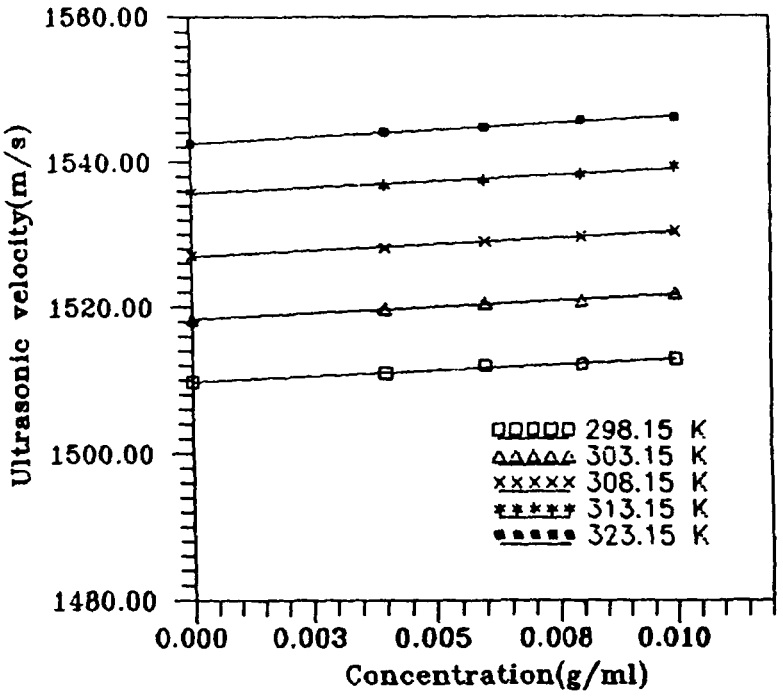

Fig 1.1(a) Plots of ultrasonic velocity versus concentration for ovalbumin at pH 2.4

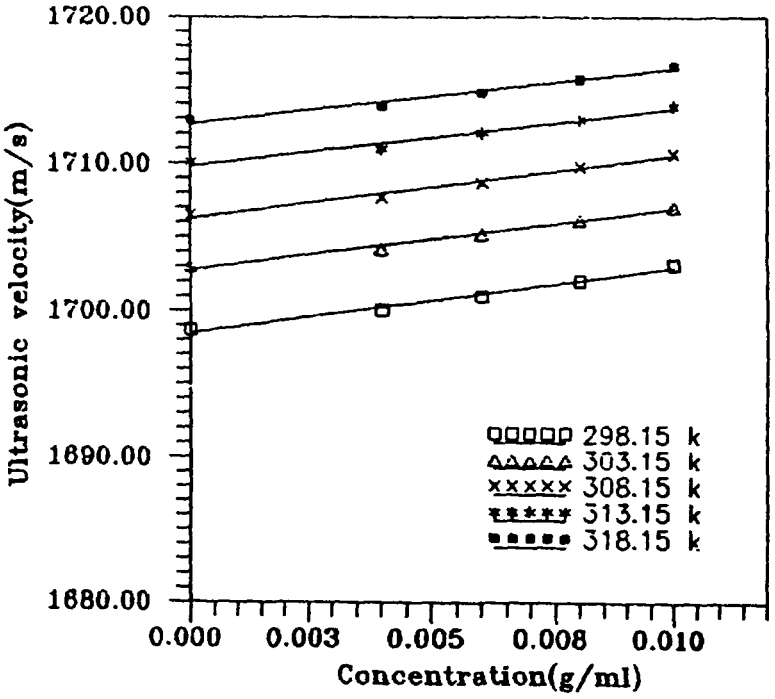

Fig 1.1(b) Plots of ultrasonic velocity versus concentration for ovalbumin–maltose system at pH 2.4

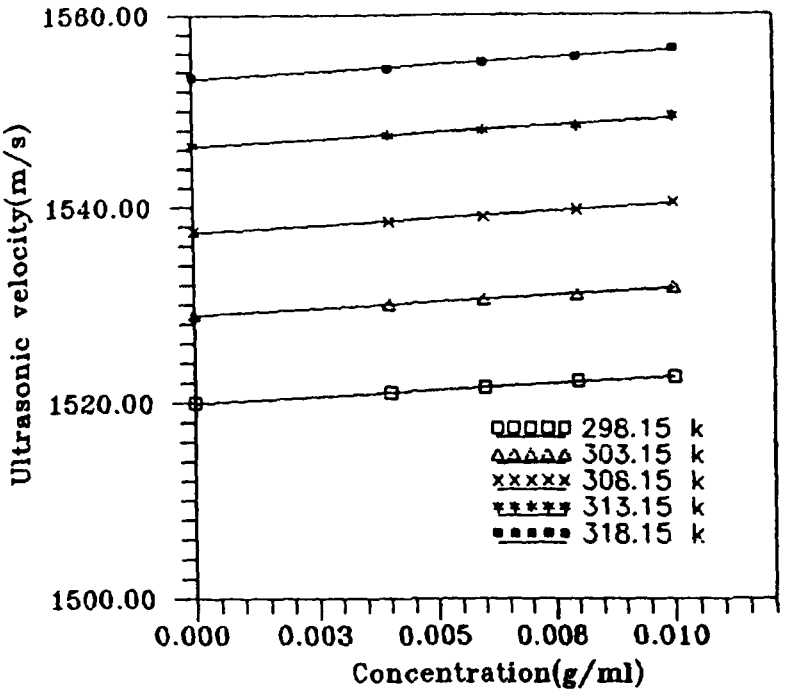

Fig 1.1(c) Plots of ultrasonic velocity versus concentration for ovalbumin at pH 7.0

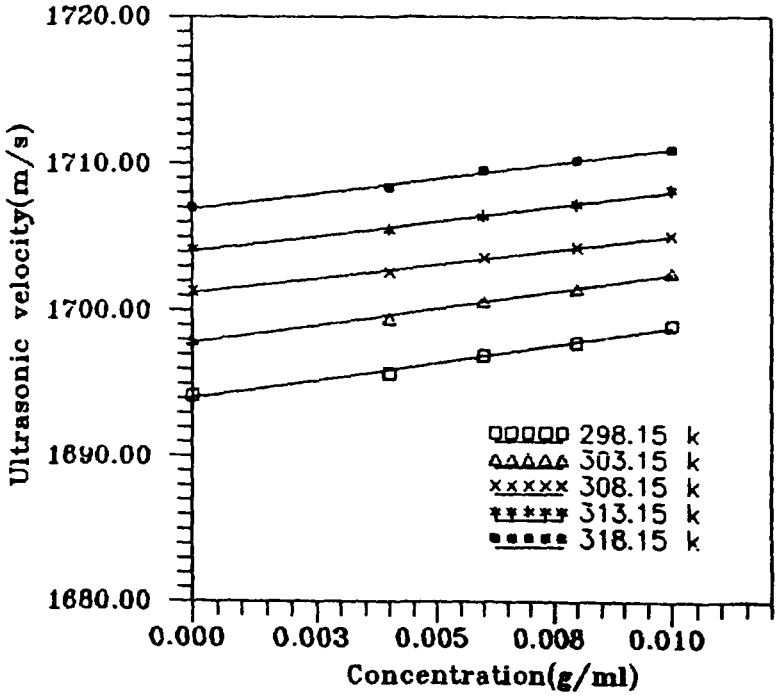

Fig 1.1(d) Plots of ultrasonic velocity versus concentration for ovalbumin–maltose system at pH 7.0

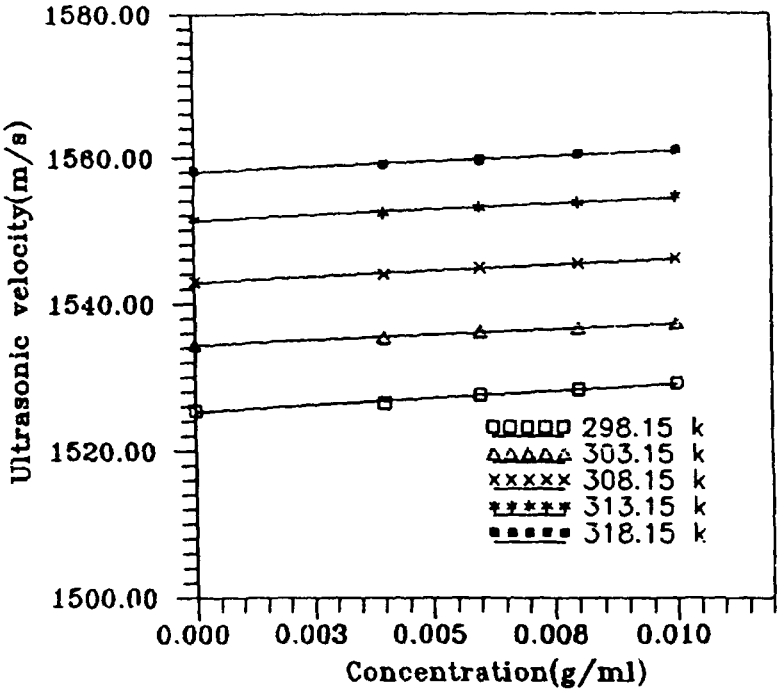

Fig 1.1(e) Plots of ultrasonic velocity versus concentration for ovalbumin at pH 8.9

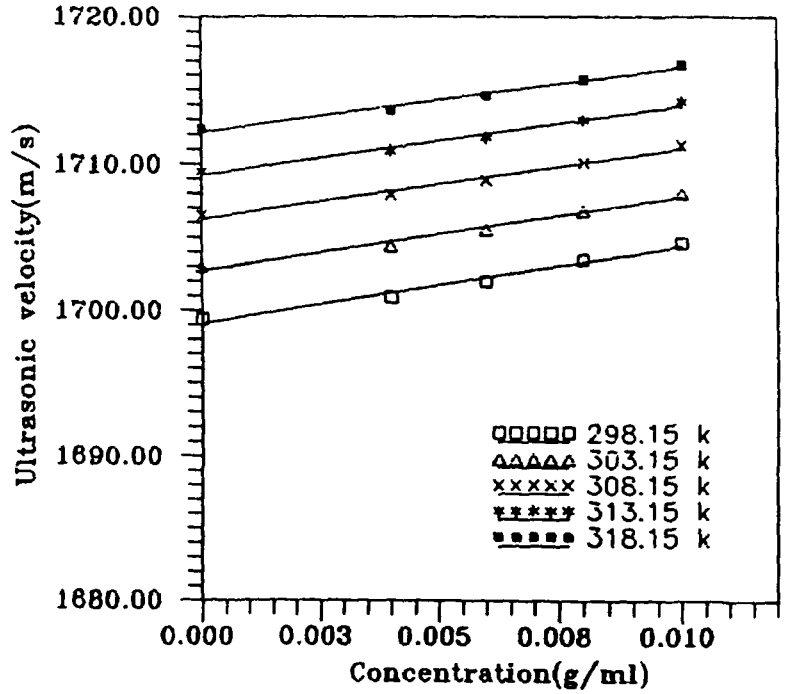

Fig 1.1(f) Plots of ultrasonic velocity versus concentration for ovalbumin–maltose system at pH 8.9

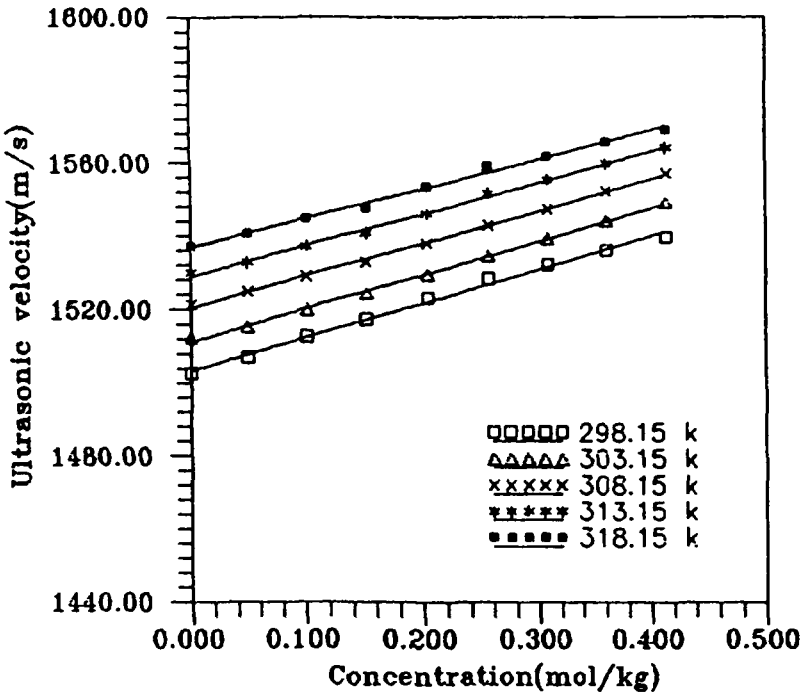

Fig 1.1(g) Plots of ultrasonic velocity versus concentration for L-valine-urea-water system

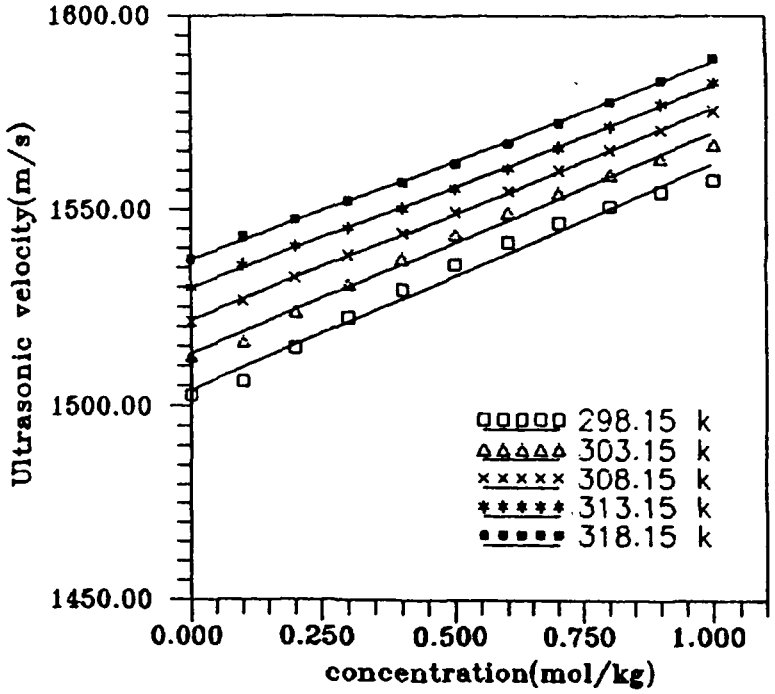

Fig 1.1(h) Plots of ultrasonic velocity versus concentration for L-serine-urea-water system

Table 1.5: Adiabatic Compressibility ($\beta_s x10^{10}, m^2N^{-1}$) as functions of temperature and concentration for the following systems:

(a) Ovalbumin- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	4.3573	4.3124	4.2693	4.2280	4.2029
0.004	4.3471	4.3024	4.2608	4.2206	4.1914
0.006	4.3422	4.2979	4.2536	4.2147	4.1859
0.008	4.3372	4.2915	4.2486	4.2096	4.1794
0.010	4.3310	4.2838	4.2416	4.2011	4.1751

(b) Ovalbumin-Maltose-Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	2.8831	2.8713	2.8641	2.8581	2.8559
0.004	2.8772	2.8663	2.8590	2.8534	2.8506
0.006	2.8733	2.8623	2.8550	2.8491	2.8466
0.008	2.8687	2.8583	2.8505	2.8451	2.8426
0.010	2.8646	2.8543	2.8465	2.8409	2.8384

(c) Ovalbumin- Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	4.2803	4.2347	4.1942	4.1530	4.1246
0.004	4.2705	4.2251	4.1846	4.1435	4.1163
0.006	4.2672	4.2214	4.1807	4.1396	4.1113
0.008	4.2616	4.2174	4.1760	4.1361	4.1070
0.010	4.2573	4.2113	4.1700	4.1287	4.0998

(d) ***Ovalbumin-Maltose-Buffer System (pH 7.0)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	2.9023	2.8939	2.8880	2.8840	2.8808
0.004	2.8960	2.8878	2.8823	2.8786	2.8766
0.006	2.8910	2.8832	2.8786	2.8746	2.8705
0.008	2.8862	2.8784	2.8745	2.8705	2.8664
0.010	2.8812	2.8740	2.8708	2.8663	2.8630

(e) ***Ovalbumin-Buffer System (pH 8.9)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	4.2378	4.1932	4.1533	4.1156	4.0884
0.004	4.2318	4.1858	4.1438	4.1069	4.0818
0.006	4.2245	4.1794	4.1383	4.1020	4.0778
0.008	4.2167	4.1750	4.1334	4.0971	4.0708
0.010	4.2106	4.1701	4.1280	4.0908	4.0661

(f) ***Ovalbumin-Maltose-Buffer System (pH 8.9)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	2.8807	2.8717	2.8640	2.8592	2.8559
0.004	2.8744	2.8657	2.8582	2.8537	2.8508
0.006	2.8696	2.8606	2.8534	2.8491	2.8460
0.008	2.8633	2.8556	2.8486	2.8442	2.8416
0.010	2.8583	2.8507	2.8439	2.8397	2.8376

(g) ***L-Valine-Urea-Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.0000	4.4338	4.3840	4.3408	4.3008	4.2702
0.0502	4.3929	4.3528	4.3065	4.2704	4.2355
0.1008	4.3558	4.3215	4.2779	4.2410	4.2081
0.1520	4.3235	4.2909	4.2510	4.2151	4.1859
0.2035	4.2849	4.2565	4.2171	4.1799	4.1489
0.2555	4.2491	4.2221	4.1832	4.1452	4.1140
0.3081	4.2222	4.1905	4.1536	4.1192	4.0935
0.3610	4.1941	4.1572	4.1213	4.0889	4.0671
0.4144	4.1684	4.1239	4.0889	4.0604	4.0429

(h) ***L-Serine-Urea-Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.1001	4.3877	4.3331	4.2788	4.2360	4.2048
0.2002	4.3177	4.2704	4.2279	4.1920	4.1637
0.3004	4.2555	4.2135	4.1792	4.1487	4.1213
0.4005	4.2004	4.1609	4.1321	4.1057	4.0793
0.5006	4.1492	4.1119	4.0863	4.0620	4.0374
0.6007	4.1013	4.0654	4.0421	4.0196	3.9962
0.7009	4.0610	4.0243	3.9998	3.9776	3.9542
0.8010	4.0213	3.9837	3.9579	3.9348	3.9122
0.9014	3.9887	3.9487	3.9178	3.8922	3.8708
1.0012	3.9596	3.9161	3.8792	3.8500	3.8289

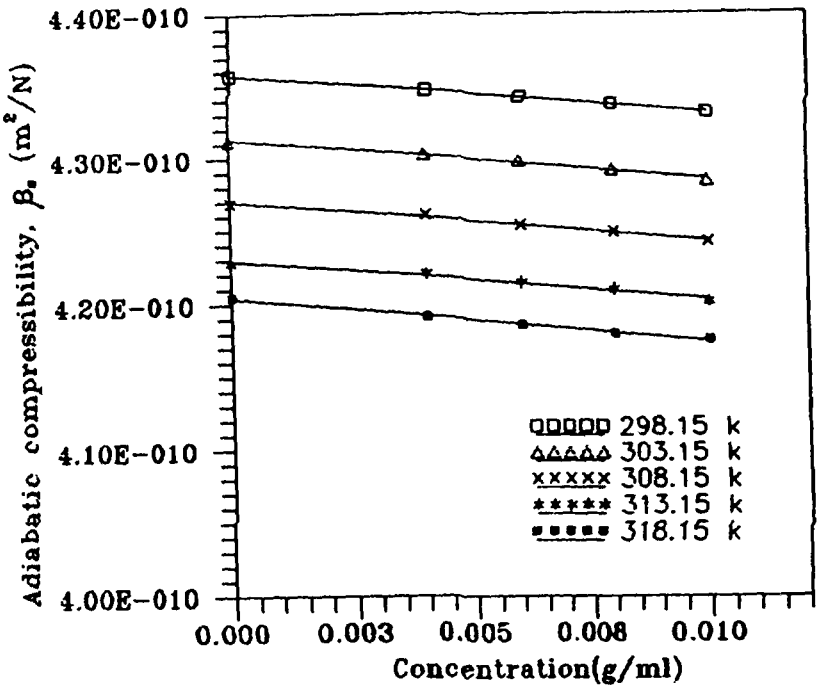

Fig 1.2(a) Plots of adiabatic compressibility versus concentration for ovalbumin at pH 2.4

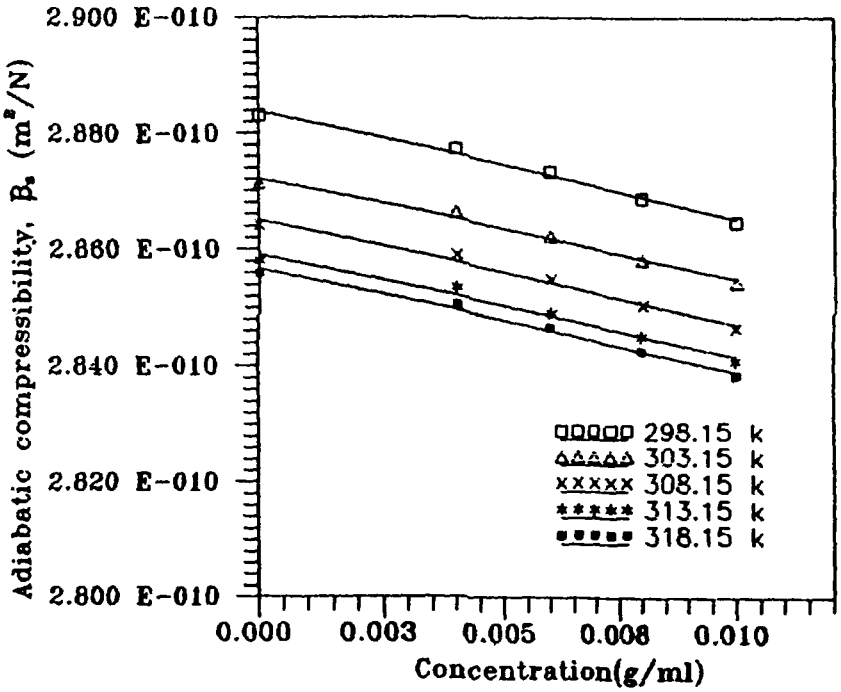

Fig 1.2(b) Plots of adiabatic compressibility versus concentration for ovalbumin–maltose system at pH 2.4

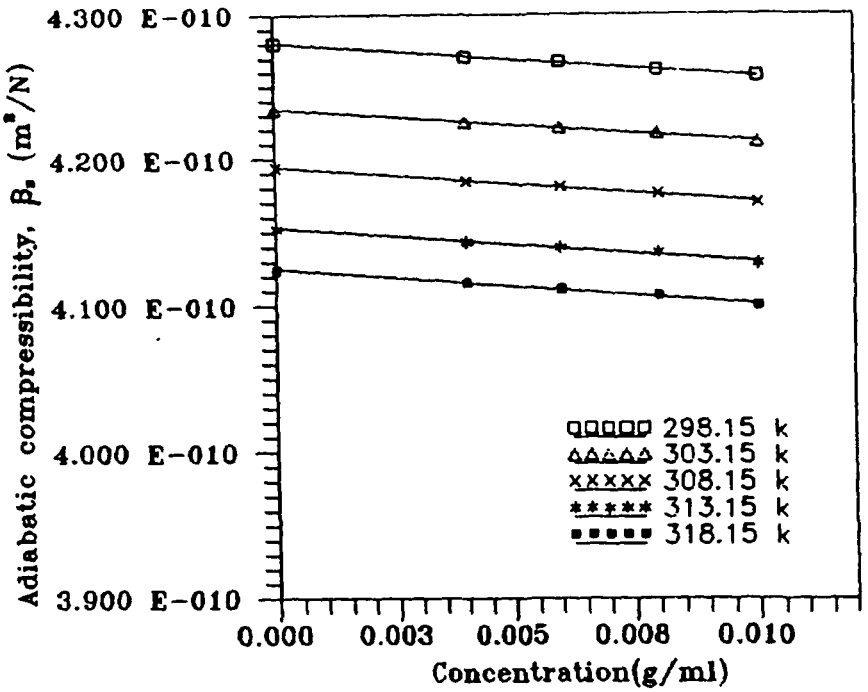

Fig 1.2(c) Plots of adiabatic compressibility versus concentration for ovalbumin at pH 7.0

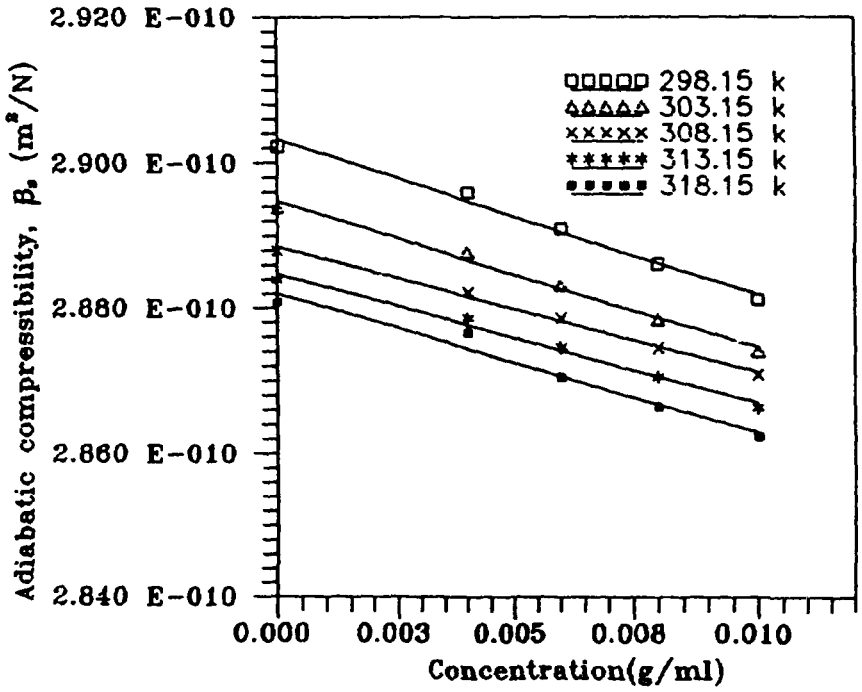

Fig 1.2(d) Plots of adiabatic compressibility versus concentration for ovalbumin–maltose system at pH 7.0

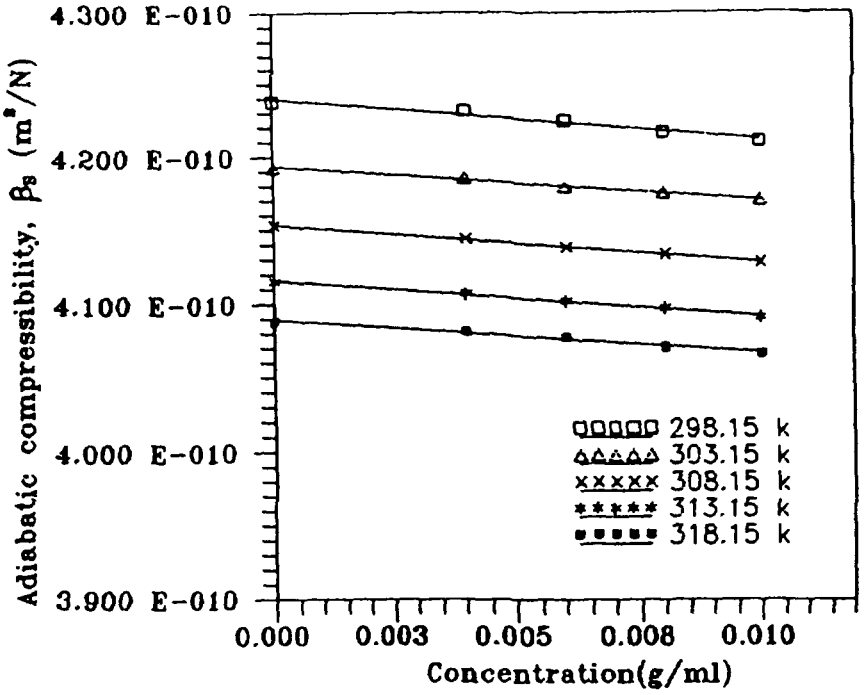

Fig 1.2(e) Plots of adiabatic compressibility versus concentration for ovalbumin at pH 8.9

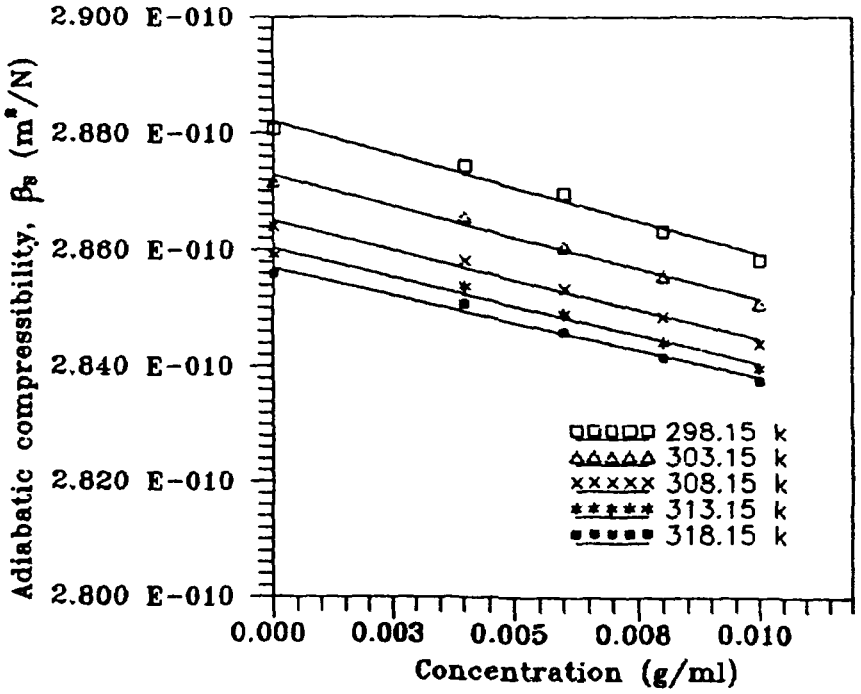

Fig 1.2(f) Plots of adiabatic compressibility versus concentration for ovalbumin–maltose system at pH 8.9

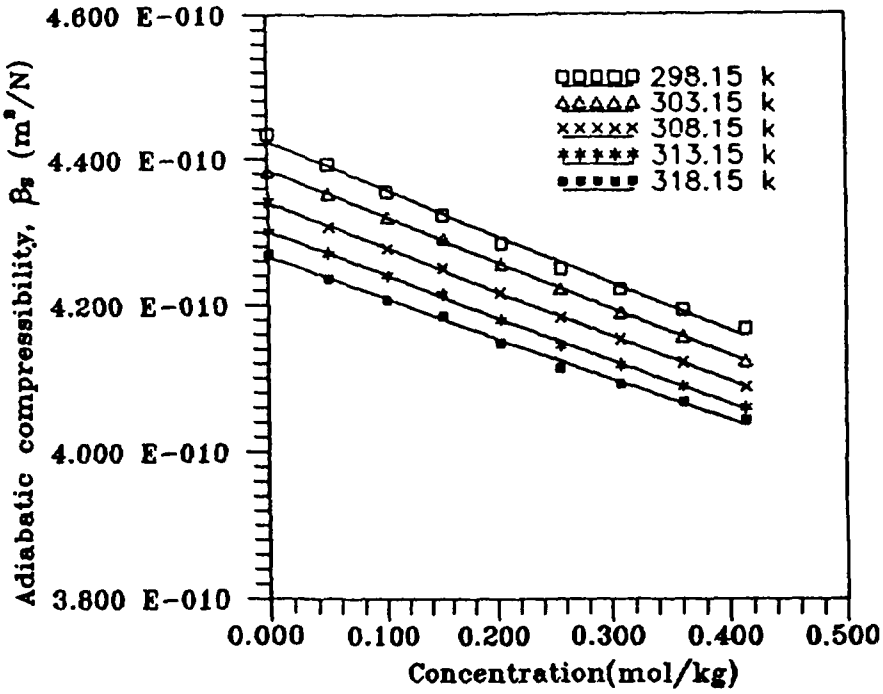

Fig 1.2(g) Plots of adiabatic compressibility versus concentration for L-valine-urea-water system

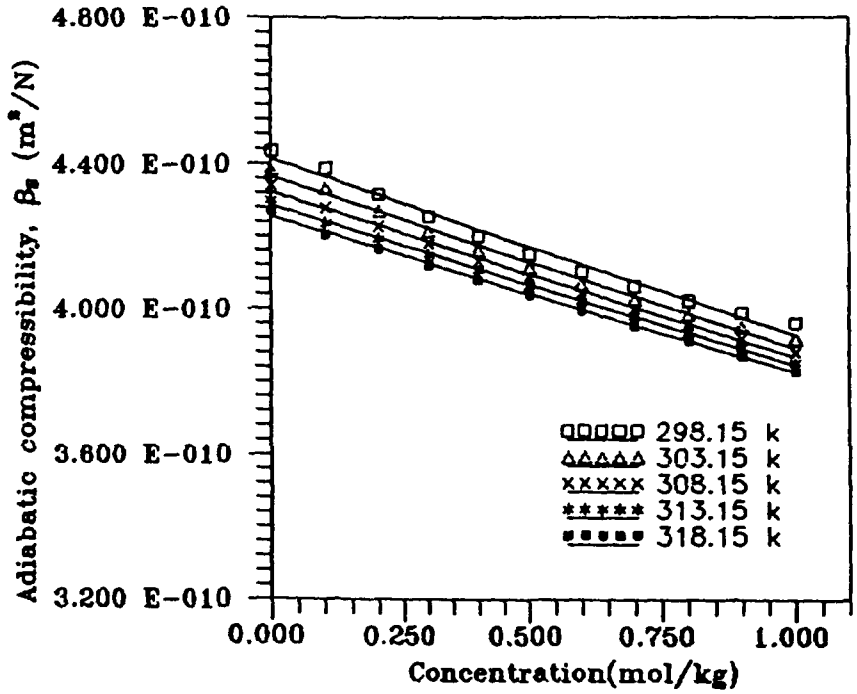

Fig 1.2(h) Plots of adiabatic compressibility versus concentration for L-serine-urea-water system

When urea is added to water it disrupts the hydrogen bonding among water molecules by dissolving exclusively within the dense or less structured phase (ice-like component) of liquid water and without inducing any change on the alternate type of long range (close-packed) structure [132-134]. This ultimately leads to an overall close-packed structure (tetrahedral type), therefore, the adiabatic compressibility of urea-water mixture is less than that of pure water.

The disruptive influence of urea on the water-water hydrogen bonding modifies the cavity size distribution, as suggested by Ben Naim [135], which solubilizes the inert solutes and rare gases. On the other hand, the dielectric constant of urea-water mixtures is similar to that of pure water, so the electrostatic solvation effects of spherical ions should be comparable in both solvents. Since amino acids exist as zwitter ions in both solvents they are also solvated by electrostatic solvation in urea-water mixtures. The structure of water in aqueous solutions of amino acids with non-polar side chains is enhanced and the probability of hydrogen bonding between water molecules is increased by forming water clathrate around the hydrophobic moiety. On the other hand, the structure of water in the solutions of amino acids with polar or charged side chains is destroyed due to electrostatic interactions or hydrogen bonding between the amino acids and the water molecules.

There are two possible explanations for the effect of side chains on the structure of water [136]:

(a) Hydration shells of NH_3^+ and COO^- groups overlap that of the side chains because these charged groups strongly disrupt the structure of water upto a considerable distance. As a result the interaction between the side chain and the water molecules is hidden by the two ionic groups.

(b) The accessibility of water to side chains is largely reduced by the self-association of amino acid molecules due to electrostatic and/or hydrophobic interactions at neutral pH. This self-association of hydrophobic R groups of valine

molecules may be responsible for the slight decrease in compressibility of the solution of L-valine when compared to that of L-serine which has polar side chain. But this effect of the side chains on the structure of water is very little [136].

As the concentration of the solution increases, there is a corresponding increase in the number of incompressible solute molecules, which causes a decrease in the compressibility of the solution.

In case of ovalbumin-maltose-buffer systems, the compressibility is reduced to just half of that of the ovalbumin-buffer systems.This great reduction is due to the increasing interactions (hydrophobic, electrostatic and hydrogen bonding) in the protein molecules in the presence of sugar. The increase in hydrophobic interactions results in the more compact structure of the stabilized protein ultimately reducing the adiabatic compressibility of the solution.

The decrease in compressibility values with increasing temperature may be caused due to the rupturing of solvent molecules [137] leading to a greater attraction among the molecules of the solution. The increase in temperature also causes a change in the solvation of molecules, which effects the compressibility values.

The pH of the solution has also shown to effect the compressibility behaviour. The increase in pH causes an increase in the ultrasonic velocity of the solution and therefore, decreases the compressibility. (Table 1.2)

$\Delta\beta$, calculated from equation 1.2, also varies with the concentration and the temperature. It increases with concentration and decreases with temperature. The variation of β_r with concentration is shown in figures 1.3 a-h. The pattern of variation of β_r is similar to that of $\Delta\beta$. Linear relationship is found between β_r and the solute concentration. The plots of relative change in compressibility (β_r) as a function of concentration show that the intercepts are in the vicinity of zero in the systems (i) and (iii) indicating the presence of weak interactions due to extremely dilute nature of the systems. In case of system (ii), the intercepts are away from

Table 1.6: Lowering in Adiabatic Compressibility($\Delta\beta$ x 10^{10} ,m^2N^{-1}) as functions of temperature and concentration for the following systems:

(a) Ovalbumin- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.0102	0.0100	0.0085	0.0074	0.0115
0.006	0.0178	0.0145	0.0157	0.0133	0.0170
0.008	0.0201	0.0209	0.0207	0.0184	0.0235
0.010	0.0263	0.0286	0.0277	0.0269	0.0278

(b) Ovalbumin-Maltose-Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.0059	0.0050	0.0051	0.0048	0.0045
0.006	0.0098	0.0090	0.0091	0.0090	0.0085
0.008	0.0144	0.0130	0.0136	0.0130	0.0125
0.010	0.0185	0.0170	0.0176	0.0172	0.0167

(c) Ovalbumin- Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.0098	0.0096	0.0096	0.0095	0.0083
0.006	0.0131	0.0133	0.0135	0.0134	0.0133
0.008	0.0187	0.0173	0.0182	0.0169	0.0177
0.010	0.0230	0.0234	0.0242	0.0243	0.0249

(d) Ovalbumin-Maltose-Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.0063	0.0061	0.0057	0.0054	0.0052
0.006	0.0113	0.0107	0.0097	0.0094	0.0103
0.008	0.0161	0.0155	0.0135	0.0135	0.0144
0.010	0.0211	0.0199	0.0172	0.0177	0.0178

(e) Ovalbumin- Buffer System (pH 8.9)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.0060	0.0074	0.0095	0.0087	0.0066
0.006	0.0133	0.0138	0.0150	0.0136	0.0106
0.008	0.0211	0.0182	0.0198	0.0185	0.0176
0.010	0.0271	0.0231	0.0252	0.0248	0.0223

(f) Ovalbumin-Maltose-Buffer System (pH 8.9)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.0063	0.0060	0.0058	0.0055	0.0051
0.006	0.0111	0.0110	0.0106	0.0101	0.0099
0.008	0.0174	0.0161	0.0154	0.0150	0.0143
0.010	0.0224	0.0210	0.0201	0.0195	0.0183

(g) ***L-Valine-Urea-Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.0502	0.0409	0.0312	0.0343	0.0304	0.0347
0.1008	0.0781	0.0625	0.0629	0.0598	0.0621
0.1520	0.1103	0.0931	0.0898	0.0857	0.0843
0.2035	0.1489	0.1275	0.1237	0.1209	0.1213
0.2555	0.1847	0.1619	0.1577	0.1556	0.1562
0.3081	0.2116	0.1935	0.1872	0.1816	0.1767
0.3610	0.2397	0.2268	0.2195	0.2118	0.2031
0.4144	0.2654	0.2601	0.2519	0.2404	0.2273

(h) ***L-Serine-Urea-Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.1001	0.0461	0.0509	0.0620	0.0648	0.0654
0.2002	0.1161	0.1136	0.1129	0.1088	0.1065
0.3004	0.1783	0.1705	0.1616	0.1521	0.1489
0.4005	0.2334	0.2231	0.2088	0.1951	0.1909
0.5006	0.2846	0.2721	0.2545	0.2388	0.2328
0.6007	0.3325	0.3186	0.2987	0.2812	0.2740
0.7009	0.3728	0.3597	0.3410	0.3232	0.3160
0.8010	0.4125	0.4002	0.3830	0.3660	0.3580
0.9014	0.4451	0.4353	0.4231	0.4086	0.3994
1.0012	0.4742	0.4679	0.4616	0.4508	0.4413

Table 1.7: Relative Lowering in Compressibility(β_r x 10^3) as functions of temperature and concentration for the following systems:

(a) Ovalbumin- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	2.3409	2.3189	1.9910	1.7502	2.7362
0.006	4.0850	3.3624	3.6774	3.1456	4.0445
0.008	4.6129	4.8465	4.8486	4.3519	5.5913
0.010	6.0358	6.6320	6.4882	6.3623	6.6145

(b) Ovalbumin-Maltose- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	2.0464	1.7414	1.7807	1.6794	1.5761
0.006	3.3991	3.1345	3.1773	3.1489	2.9771
0.008	4.9946	4.5276	4.7484	4.5485	4.3781
0.010	6.4167	5.9207	6.1450	6.0180	5.8492

(c) Ovalbumin-Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	2.2896	2.2670	2.2888	2.2875	2.0123
0.006	3.0605	3.1407	3.2187	3.2266	3.2246
0.008	4.3689	4.0853	4.3394	4.0693	4.2913
0.010	5.3735	5.5258	5.7699	5.8512	6.0369

(d) ***Ovalbumin-Maltose-Buffer System*** *(pH 7.0)*

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	2.1707	2.1079	1.9737	1.8724	1.8051
0.006	3.8935	3.6974	3.3587	3.2594	3.5754
0.008	5.5473	5.3561	4.6745	4.6810	4.9986
0.010	7.2701	6.8765	5.9557	6.1373	6.1788

(e) ***Ovalbumin-Buffer System*** *(pH 8.9)*

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	1.4158	1.7648	2.2873	2.1139	1.6143
0.006	3.1384	3.2910	3.6116	3.3045	2.5927
0.008	4.9790	4.3404	4.7673	4.4951	4.3049
0.010	6.3948	5.5089	6.0675	6.0259	5.4545

(f) ***Ovalbumin-Maltose-Buffer System*** *(pH 8.9)*

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	2.1870	2.0894	2.0251	1.9236	1.7858
0.006	3.8532	3.8305	3.7011	3.5325	3.4665
0.008	6.0402	5.6064	5.3771	5.2462	5.0072
0.010	7.7759	7.3127	7.0182	6.8201	6.4078

(g) ***L-Valine-Urea-Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.0502	09.2359	07.1098	07.9052	07.0789	08.1283
0.1008	17.6039	14.2614	14.5017	13.9124	14.5465
0.1520	24.8781	21.2452	20.6851	19.9194	19.7410
0.2035	33.5748	29.0865	28.4984	28.1146	28.4104
0.2555	41.6643	36.9379	36.3227	36.1881	36.5766
0.3081	47.7353	44.1409	43.1297	42.2300	41.3700
0.3610	54.0649	51.7333	50.5776	49.2640	47.5661
0.4144	59.8496	59.3366	58.0401	55.8931	53.2260

(h) ***L-Serine-Urea-Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.1001	10.3888	11.6152	14.2903	15.0744	15.3113
0.2002	26.1819	25.9133	26.0114	25.2924	24.9343
0.3004	40.2230	38.8910	37.2315	35.3688	34.8721
0.4005	52.6326	50.8821	48.0926	45.3639	44.7028
0.5006	64.1897	62.0589	58.6402	55.5283	54.5208
0.6007	74.9902	72.6728	68.8215	65.3937	64.1676
0.7009	84.9668	83.0121	79.7017	76.3000	75.1498
0.8010	93.0342	91.2990	88.2215	85.0929	83.8305
0.9014	100.3916	99.2865	97.4600	95.0085	93.5392
1.0012	106.9526	106.7244	106.3470	104.8081	103.3371

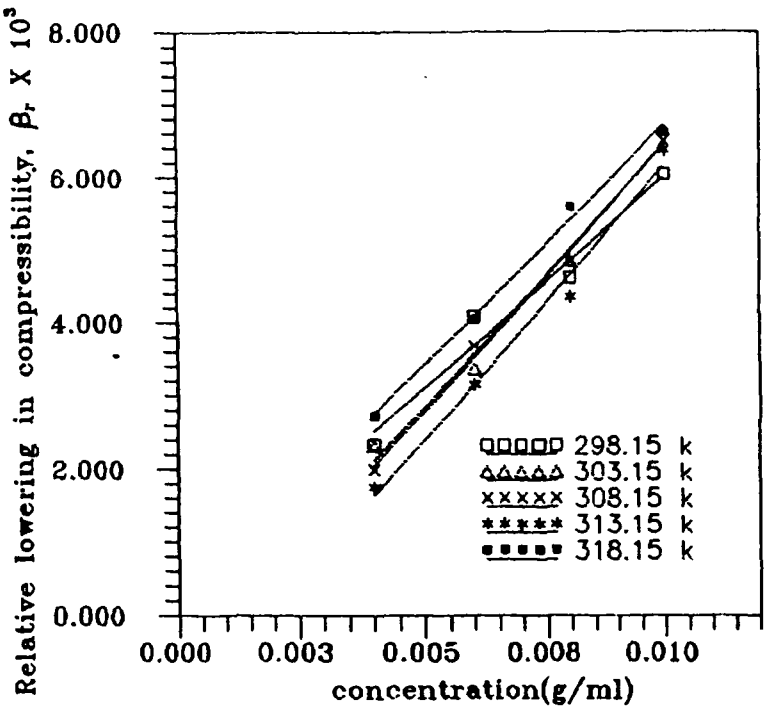

Fig 1.3(a) Plots of relative lowering in compress-ibility versus concentration for ovalbumin at pH 2.4

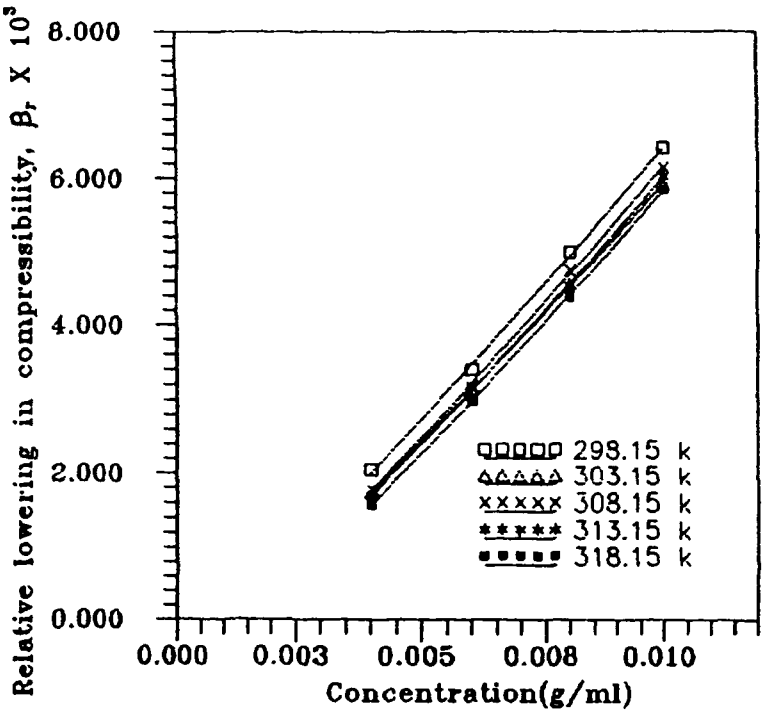

Fig 1.3(b) Plots of relative lowering in compressibility versus concentration for ovalbumin–maltose system at pH 2.4

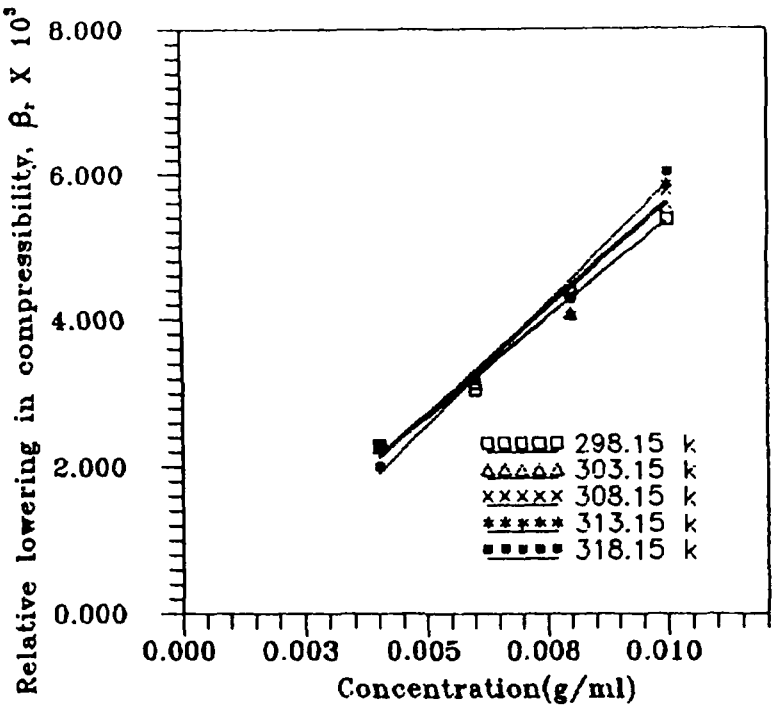

Fig 1.3(c) Plots of relative lowering in compress-ibility versus concentration for ovalbumin at pH 7.0

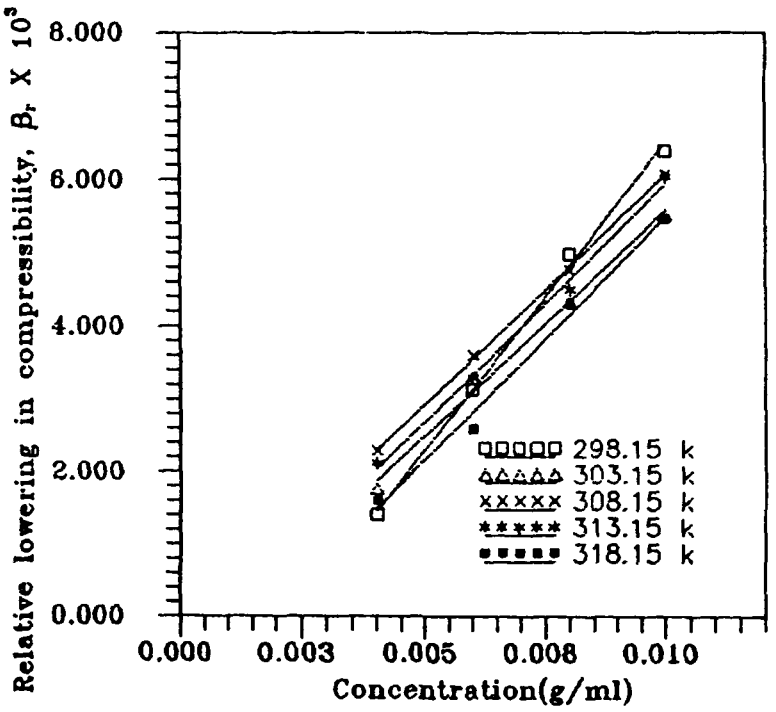

Fig 1.3(d) Plots of relative lowering in compressibility versus concentration for ovalbumin–maltose system at pH 7.0

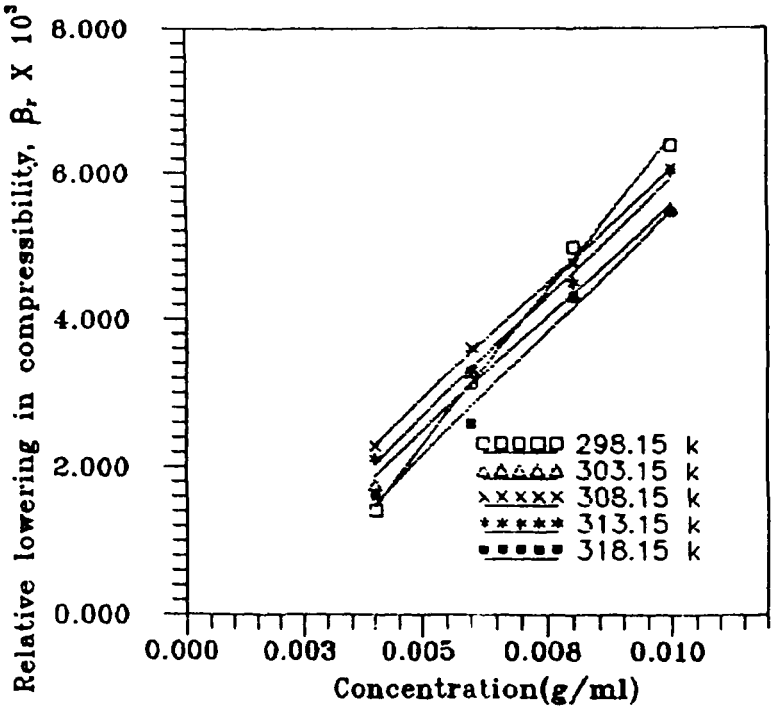

Fig 1.3(e) Plots of relative lowering in compress-ibility versus concentration for ovalbumin at pH 8.9

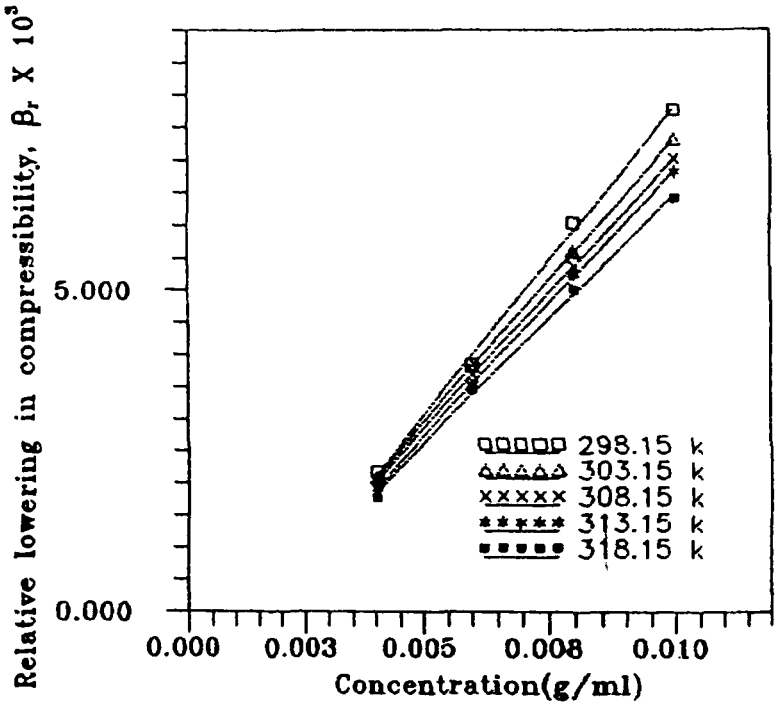

Fig 1.3(f) Plots of relative lowering in compressibility versus concentration for ovalbumin–maltose system at pH 8.9

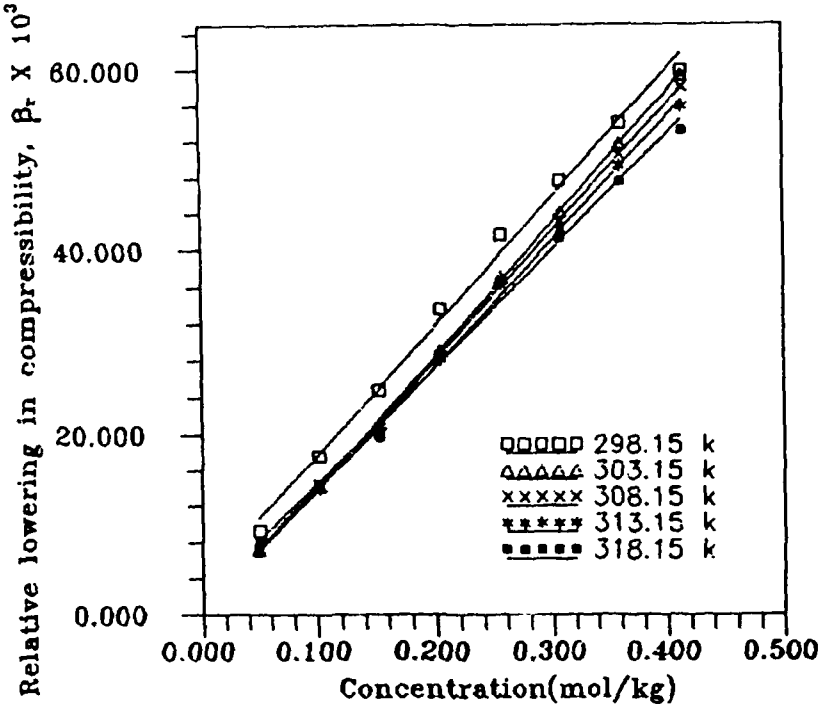

Fig 1.3(g) Plots of relative lowering in compressibility versus concentration for L–valine–urea–water system

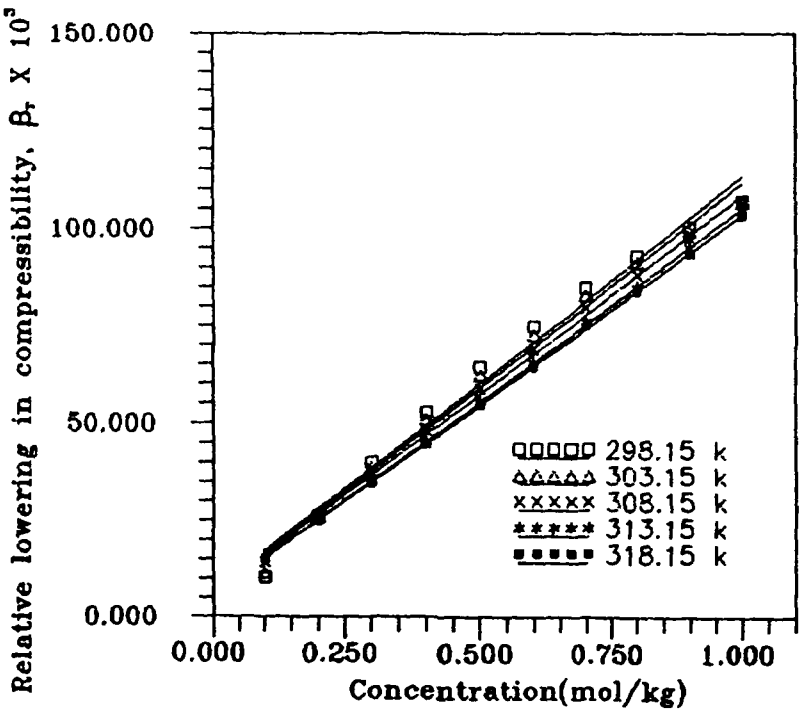

Fig 1.3(h) Plots of relative lowering in compressibility versus concentration for L–serine–urea–water system

zero indicating the presence of strong interactions (strengthening of hydrophobic interactions) due to the presence of sugar maltose.

As shown in figures 1.4 and 1.5, respectively, the specific acoustic impedance, Z and the molar sound velocity, R, both increase with an increase in temperature. This is in accordance with equations 1.4 and 1.5, respectively, in which Z is directly proportional to the ultrasonic velocity and R is proportional to the cube root of ultrasonic velocity, which increases with an increase in temperature. The increase in the value of Z and R, with an increase in the concentration of the solutions is also depicted in figures 1.4 and 1.5, respectively. An examination of tables 1.10 a and b shows that Wada's constant also increases with an increase in temperature as well as concentration.

All the parameters evaluated here vary linearly with the concentration of the solutes.

Table 1.8: Specific Acoustic Impedance ($Zx10^{-5}$kg $m^{-2}s^{-1}$) as functions of temperature and concentration for the following systems:

(a) Ovalbumin- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	15.2007	15.2721	15.3382	15.3984	15.4250
0.004	15.2253	15.2968	15.3589	15.4172	15.4524
0.006	15.2359	15.3064	15.3756	15.4319	15.4655
0.008	15.2480	15.3221	15.3878	15.4435	15.4807
0.010	15.2626	15.3398	15.4050	15.4638	15.4925

(b) Ovalbumin-Maltose-Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	20.4184	20.4496	20.4609	20.4596	20.4469
0.004	20.4438	20.4709	20.4822	20.4824	20.4691
0.006	20.4596	20.4870	20.4993	20.5007	20.4850
0.008	20.4797	20.5051	20.5176	20.5183	20.5043
0.010	20.4997	20.5228	20.5370	20.5377	20.5237

(c) Ovalbumin- Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	15.3702	15.4444	15.5072	15.5702	15.6065
0.004	15.3956	15.4698	15.5312	15.5926	15.6269
0.006	15.4021	15.4780	15.5424	15.6054	15.6418
0.008	15.4153	15.4876	15.5525	15.6135	15.6525
0.010	15.4270	15.5018	15.5668	15.6314	15.6708

(d) Ovalbumin-Maltose-Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	20.3372	20.3505	20.3527	20.3464	20.3355
0.004	20.3637	20.3758	20.3767	20.3688	20.3578
0.006	20.3849	20.3953	20.3938	20.3864	20.3790
0.008	20.4076	20.4180	20.4124	20.4062	20.3993
0.010	20.4300	20.4363	20.4288	20.4249	20.4139

(e) Ovalbumin- Buffer System (pH 8.9)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	15.4686	15.5414	15.6044	15.6608	15.6973
0.004	15.4802	15.5597	15.6237	15.6798	15.7147
0.006	15.4960	15.5755	15.6427	15.6977	15.7239
0.008	15.5163	15.5868	15.6449	15.7095	15.7429
0.010	15.5306	15.5990	15.6682	15.7253	15.7552

(f) Ovalbumin-Maltose-Buffer System (pH 8.9)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	20.4268	20.4479	20.4609	20.4593	20.4478
0.004	20.4533	20.4733	20.4846	20.4829	20.4707
0.006	20.4751	20.4967	20.5068	20.5039	20.4929
0.008	20.5016	20.5174	20.5280	20.5252	20.5112
0.010	20.5282	20.5387	20.5476	20.5430	20.5283

(g) ***L-Valine-Urea-Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.0000	15.0090	15.0811	15.1430	15.1980	15.2363
0.0502	15.1037	15.1601	15.2277	15.2774	15.3233
0.1008	15.1769	15.2248	15.2885	15.3403	15.3831
0.1520	15.2418	15.2882	15.3459	15.3972	15.4347
0.2035	15.3224	15.3613	15.4190	15.4728	15.5143
0.2555	15.3992	15.4361	15.4938	15.5491	15.5916
0.3081	15.4589	15.5049	15.5597	15.6090	15.6414
0.3610	15.5228	15.5784	15.6322	15.6791	15.7040
0.4144	15.5828	15.6537	15.7065	15.7458	15.7626

(h) ***L-Serine-Urea-Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.1001	15.1313	15.2211	15.3082	15.3724	15.4130
0.2002	15.2914	15.3675	15.4338	15.4851	15.5199
0.3004	15.4356	15.5039	15.5558	15.5983	15.6322
0.4005	15.5662	15.6323	15.6752	15.7107	15.7444
0.5006	15.6928	15.7561	15.7946	15.8278	15.8580
0.6007	15.8173	15.8778	15.9118	15.9415	15.9693
0.7009	15.9228	15.9875	16.0253	16.0552	16.0846
0.8010	16.0353	16.1014	16.1414	16.1729	16.2014
0.9014	16.1279	16.2015	16.2536	16.2920	16.3180
1.0012	16.2151	16.2979	16.3642	16.4110	16.4372

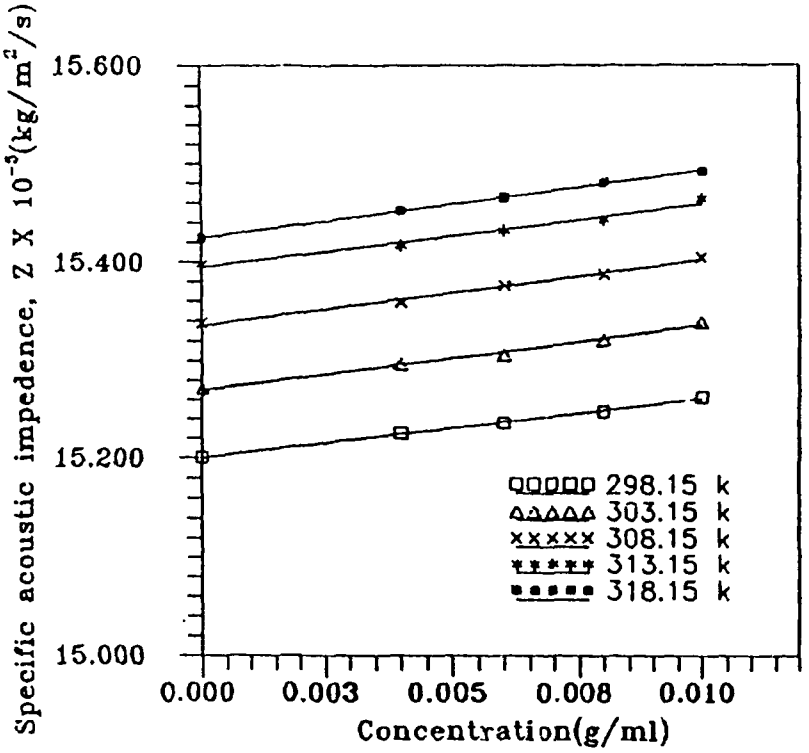

Fig 1.4(a) Plots of specific acoustic impedence versus concentration for ovalbumin at pH 2.4

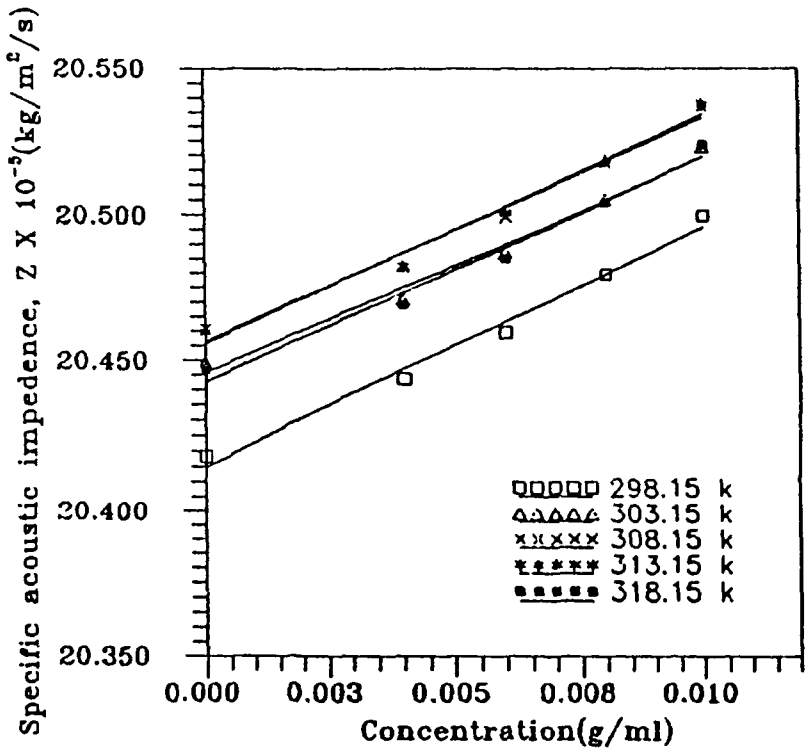

Fig 1.4(b) Plots of specific acoustic impedence versus concentration for ovalbumin–maltose system at pH 2.4

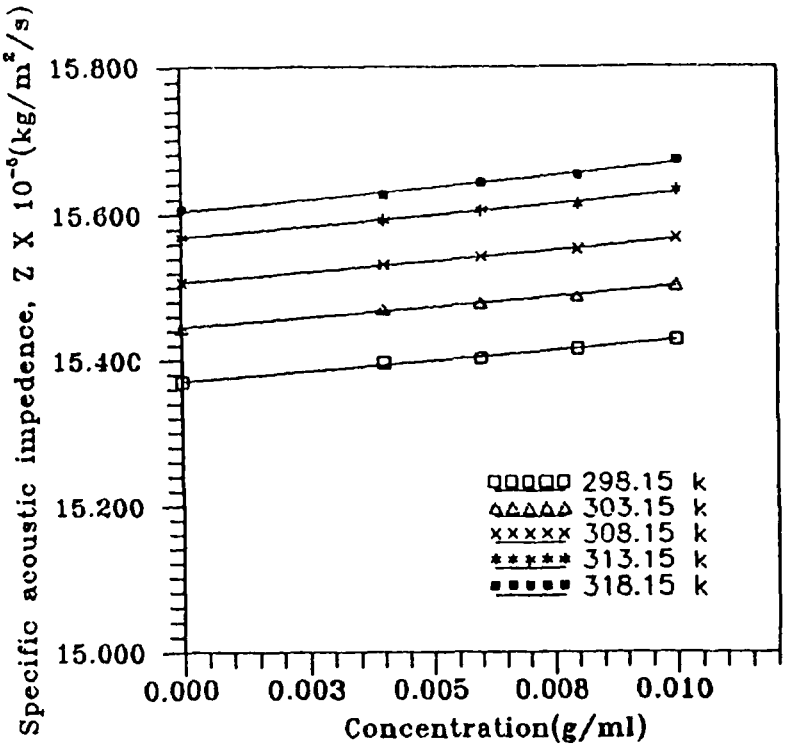

Fig 1.4(c) Plots of specific acoustic impedence versus concentration for ovalbumin at pH 7.0

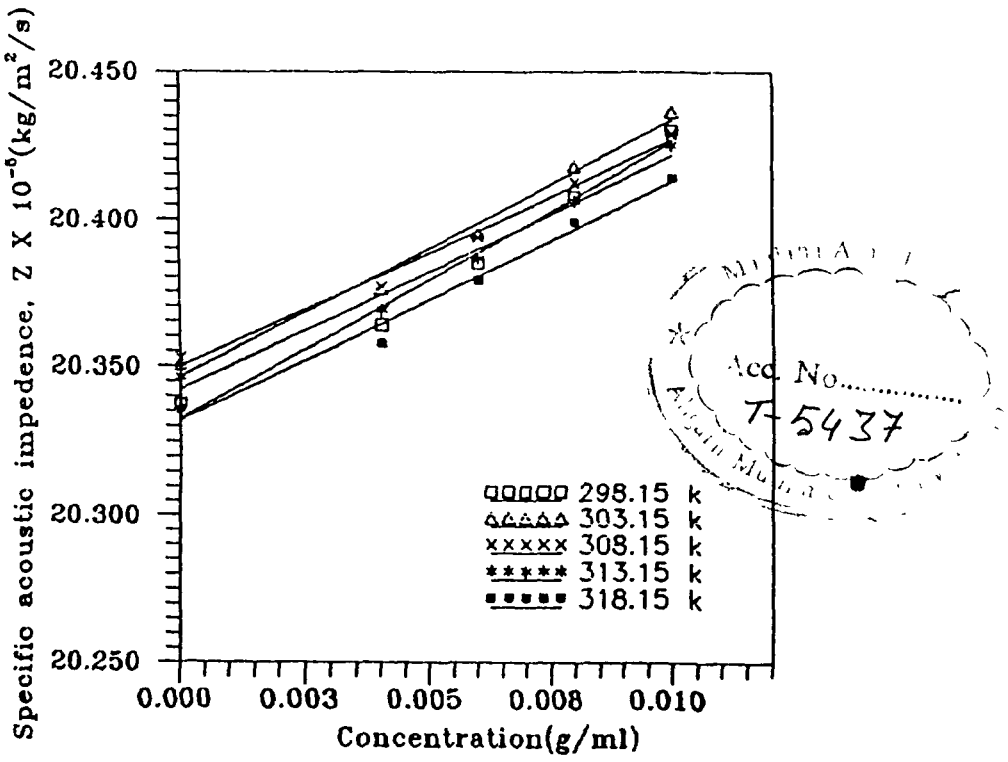

Fig 1.4(d) Plots of specific acoustic impedence versus concentration for ovalbumin–maltose system at pH 7.0

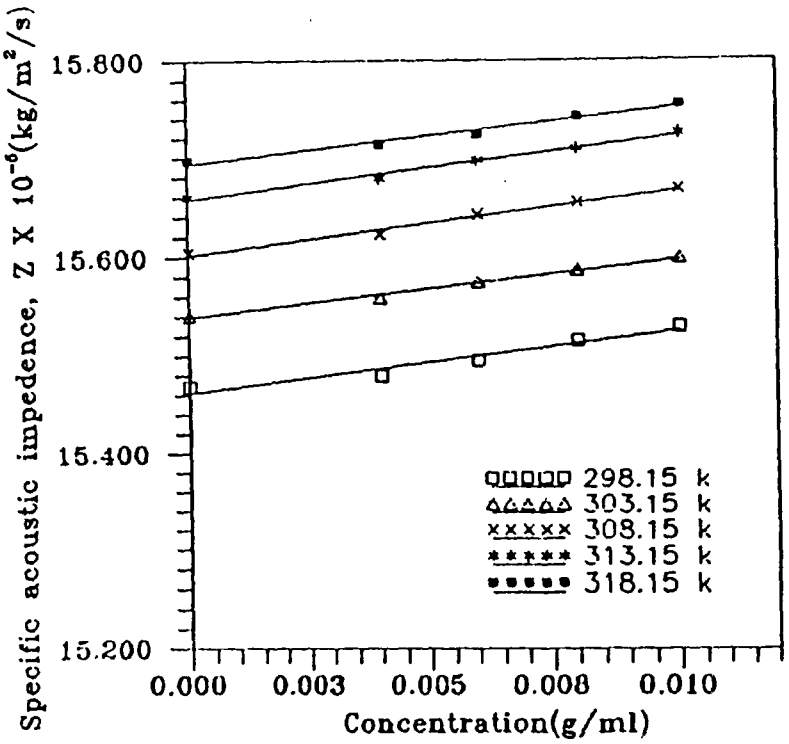

Fig 1.4(e) Plots of specific acoustic impedence versus concentration for ovalbumin at pH 8.9

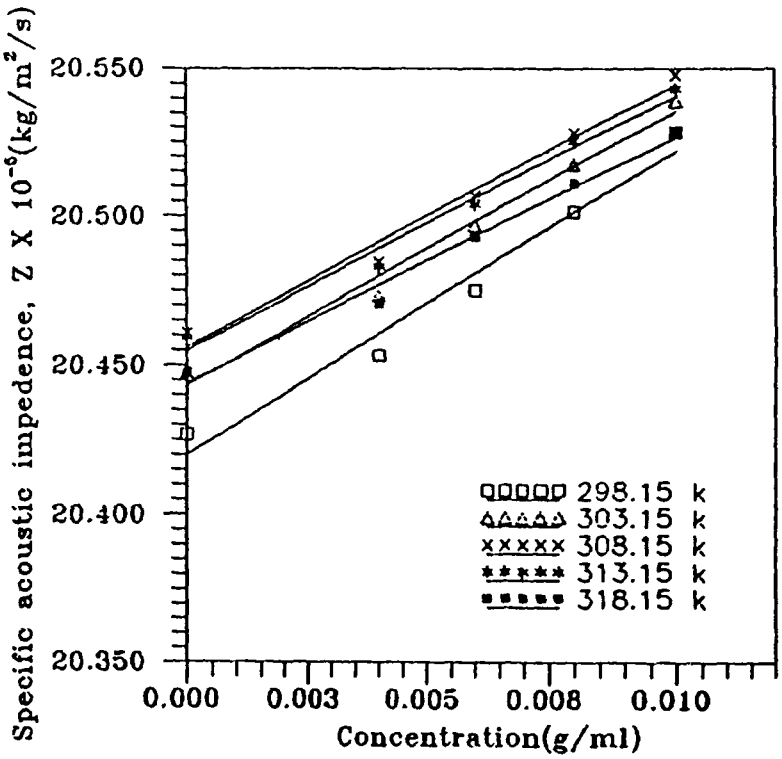

Fig 1.4(f) Plots of specific acoustic impedence versus concentration for ovalbumin–maltose system at pH 8.9

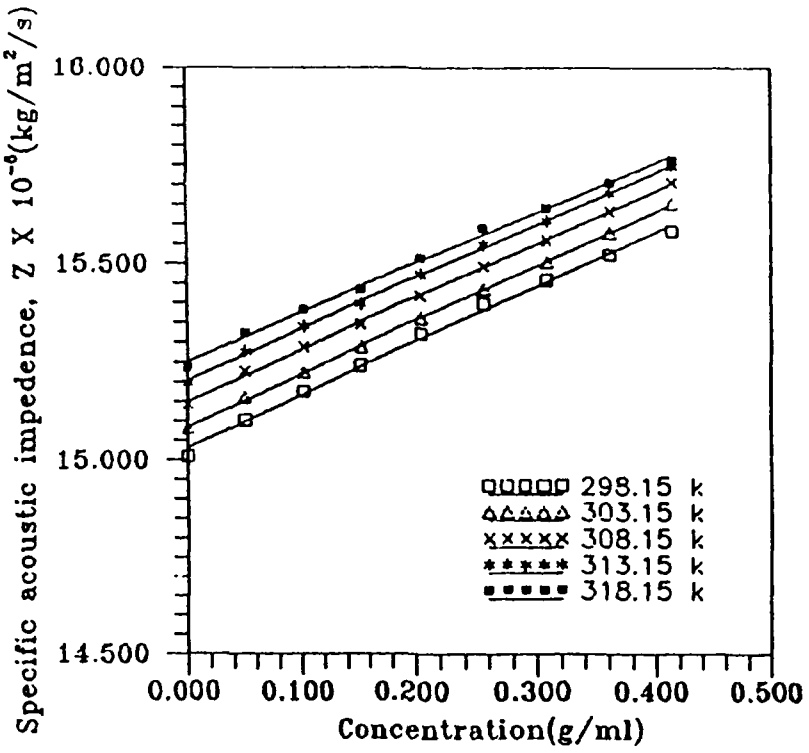

Fig 1.4(g) Plots of specific acoustic impedence versus concentration for L–valine–urea–water system

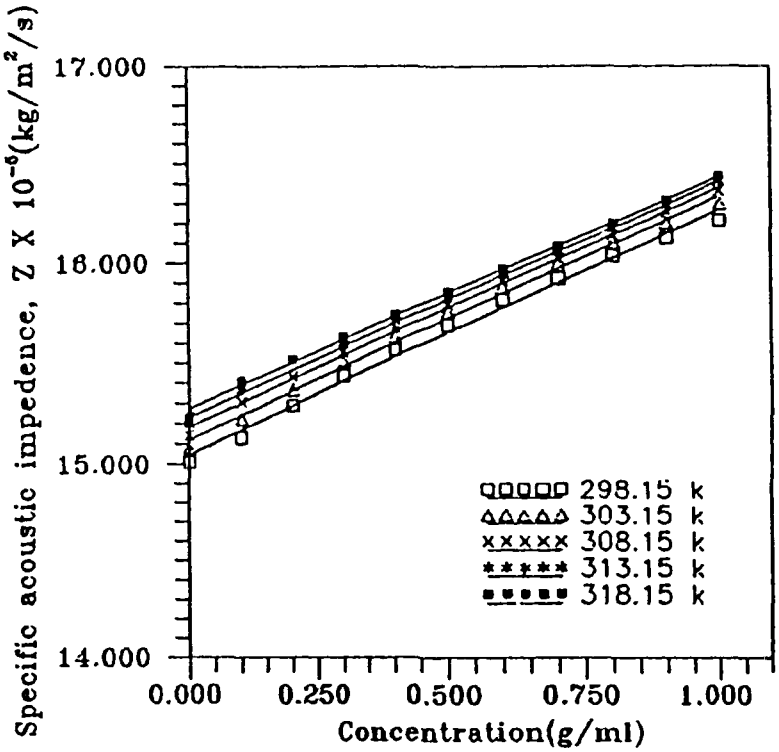

Fig 1.4(h) Plots of specific acoustic impedence versus concentration for L–serine–urea–water system

Table 1.9: Molar Sound Velocity (R, $m^{3\frac{1}{3}}\ mol^{-1}s^{-1/3}$) as functions of temperature and concentration for the following systems:

(a) ***L-Valine-Urea-Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.0502	0.2078	0.2089	0.2094	0.2102	0.2110
0.1008	0.2089	0.2096	0.2104	0.2111	0.2119
0.1520	0.2099	0.2106	0.2113	0.2121	0.2128
0.2035	0.2109	0.2115	0.2123	0.2131	0.2139
0.2555	0.2119	0.2125	0.2133	0.2141	0.2149
0.3081	0.2128	0.2135	0.2143	0.2150	0.2158
0.3610	0.2137	0.2145	0.2152	0.2160	0.2167
0.4144	0.2147	0.2155	0.2162	0.2170	0.2178

(b) ***L-Serine-Urea-Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.1001	0.2080	0.2086	0.2094	0.2101	0.2109
0.2002	0.2092	0.2098	0.2105	0.2113	0.2121
0.3004	0.2104	0.2110	0.2116	0.2124	0.2132
0.4005	0.2117	0.2122	0.2128	0.2135	0.2143
0.5006	0.2128	0.2134	0.2139	0.2146	0.2154
0.6007	0.2145	0.2151	0.2156	0.2163	0.2171
0.7009	0.2162	0.2168	0.2174	0.2181	0.2189
0.8010	0.2173	0.2179	0.2186	0.2193	0.2201
0.9014	0.2180	0.2187	0.2194	0.2201	0.2209
1.0012	0.2199	0.2206	0.2212	0.2218	0.2227

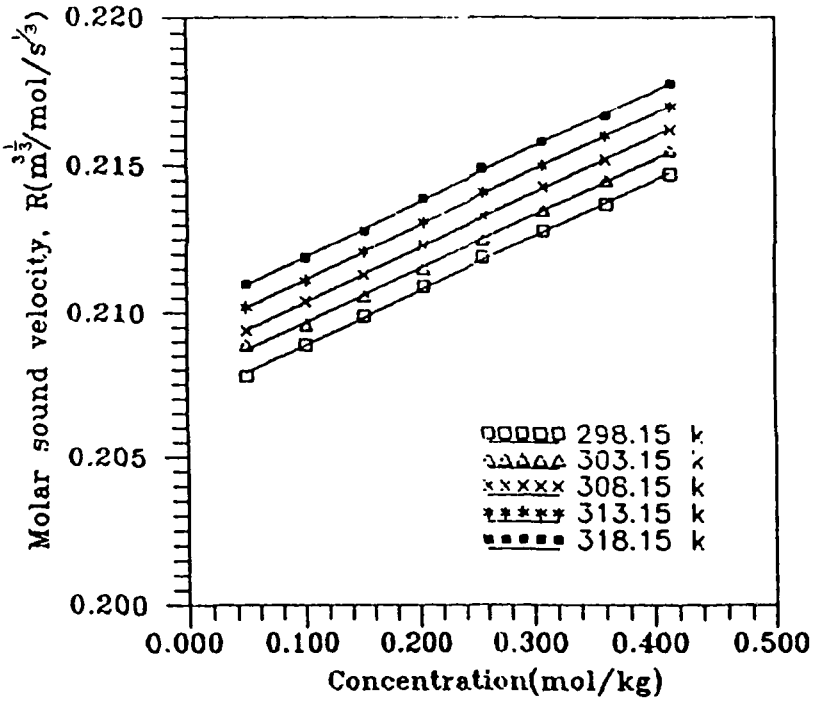

Fig 1.5(a) Plots of molar sound velocity versus concentration for L-valine-urea-water system

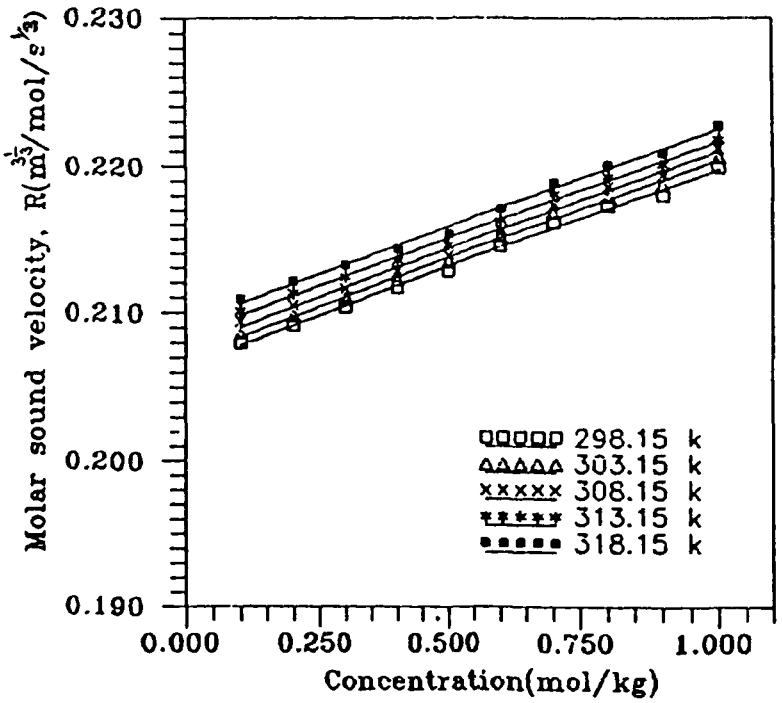

Fig 1.5(b) Plots of molar sound velocity versus concentration for L-serine-urea-water system

Table 1.10: Wadas Constant, B, as functions of temperature and concentration for the following systems:

(a) ***L-Valine-Urea-Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.0502	0.3936	0.3948	0.3961	0.3974	0.3987
0.1008	0.3956	0.3967	0.3980	0.3993	0.4006
0.1520	0.3976	0.3986	0.3999	0.4011	0.4023
0.2035	0.3995	0.4005	0.4017	0.4030	0.4043
0.2555	0.4013	0.4023	0.4036	0.4049	0.4062
0.3081	0.4032	0.4042	0.4055	0.4068	0.4080
0.3610	0.4049	0.4061	0.4074	0.4086	0.4098
0.4144	0.4067	0.4080	0.4092	0.4105	0.4116

(b) ***L-Serine-Urea-Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.1001	0.3942	0.3951	0.3963	0.3976	0.3988
0.2002	0.3965	0.3975	0.3986	0.3999	0.4012
0.3004	0.3989	0.3999	0.4010	0.4022	0.4035
0.4005	0.4015	0.4024	0.4034	0.4045	0.4058
0.5006	0.4038	0.4047	0.4056	0.4067	0.4080
0.6007	0.4064	0.4074	0.4083	0.4094	0.4107
0.7009	0.4086	0.4095	0.4104	0.4115	0.4128
0.8010	0.4106	0.4117	0.4127	0.4139	0.4151
0.9014	0.4130	0.4140	0.4150	0.4162	0.4175
1.0012	0.4146	0.4156	0.4168	0.4180	0.4193

CHAPTER -2

COMPRESSIBILITY - STRUCTURE RELATIONSHIP OF OVALBUMIN

INTRODUCTION

The volumetric and compressibility behaviour of solutes in solution provide very useful informations related to solute-solvent and solute-solute interactions. The infinite dilution partial molar volumes and compressibilities give the structural information and interaction phenomena associated with solvation processes. Since these properties are independent of solute-solute interactions, they are determined only by the respective intrinsic value and the solute-solvent interactions.

Various investigations have been done using electrolytes [138,139], carbohydrates [140,141], amino acids [51-54], peptides [13,15,66] and proteins [60-64,67] in aqueous as well as mixed aqueous solvents.

As demonstrated by X-ray analyses [142,143], the thermal fluctuations in the protein structure, due to imperfect packing and cavities, is a function of temperature and pressure. Since the fluctuation in volume is directly related to the compressibility [61-63], the effect of temperature on the compressibility is a matter of interest.

Sugar solutions have large effects on the structure and properties of proteins including their solubility, denaturation, etc. In the literature there are reports about the effect of sugars on the stability of proteins and enzymes [4-9]. Therefore, in order to study the behaviour of proteins in sugar solutions, we have studied the partial specific properties of the protein systems described earlier.

THEORY

The partial specific adiabatic compressibility of ovalbumin, $\bar{\beta}_s$, was calculated with the equation given by Shiio [144],

$$\bar{\beta}_s = -(1/\bar{v}^0)\ (\partial \bar{v}^0 / \partial P) \qquad 2.1$$

$$\bar{\beta}_s = (\beta^0 / \bar{v}^0) \lim c \to 0 \ [(\beta / \beta_0 - V_0) / c] \qquad 2.2$$

$$V_0 = (\rho - c) / \rho_0 \qquad 2.3$$

$$\bar{v}^0 = \lim c \to 0 \ [(1 - V_0) / c] \qquad 2.4$$

where P is the pressure, c is the protein concentration in grams per millilitre of the solution, V_0 is the apparent volume fraction of the solvent in solution and $\bar{v}^0$ is the partial specific volume of the protein.

RESULT AND DISCUSSION

The partial specific volume of protein at infinite dilution, $\bar{v}^0$, was determined by linear extrapolation of the apparent specific volume, $(1-V_0)/c$ to zero concentration of ovalbumin. The apparent volume fraction of ovalbumin, V_0, the apparent specific volume, $(1-V_0)/c$, and the partial specific volume of ovalbumin, are recorded in tables 2.1, 2.2 and 2.3, respectively. The adiabatic compressibility, $\bar{\beta}_s$, was determined by the linear extrapolation of $(\beta / \beta_0 - V_0)/c$ to zero protein concentration. The $\bar{\beta}_s$ values thus obtained are listed in tables 2.5 (a-f).

The partial specific volume of a protein in solution consists of three factors [10], (i) the constitutive atomic volume (V_c), (ii) the volume of the cavities formed due to imperfect packing of atoms or groups (V_{cav}), and (iii) the volume change due to solvation (ΔV_{soln}).

$$\bar{v}^0 = V_c + V_{cav} + \Delta V_{soln} \qquad 2.5$$

Here the constitutive atomic volume is considered to be highly incompressible. V_{cav} involves (i) the incompressible voids formed by the closest packing of atoms or groups and (ii) the compressible voids formed by the random close packing of atoms. ΔV_{soln} consists of three contributing effect (i) electrostatic solvation of ionic groups (ii) hydrogen bonded hydration of polar groups and (iii) hydrophobic hydration of non-polar or hydrophobic groups. Each of them contribute negatively to ΔV_{soln} [61]. Therefore, ΔV_{soln} also contributes negatively to $\bar{v}^0$ but the term

Table 2.1: Apparent Volume Fraction, V_0, of the solvent as functions of temperature and concentration for the following systems:

(a) Ovalbumin- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.9969	0.9969	0.9967	0.9967	0.9968
0.006	0.9952	0.9952	0.9952	0.9952	0.9952
0.008	0.9936	0.9937	0.9936	0.9935	0.9936
0.010	0.9921	0.9922	0.9922	0.9921	0.9921

(b) Ovalbumin- Maltose-Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.9971	0.9970	0.9970	0.9971	0.9971
0.006	0.9957	0.9956	0.9956	0.9958	0.9957
0.008	0.9943	0.9942	0.9942	0.9945	0.9945
0.010	0.9930	0.9928	0.9929	0.9932	0.9932

(c) Ovalbumin- Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.9970	0.9970	0.9969	0.9968	0.9968
0.006	0.9952	0.9952	0.9953	0.9953	0.9953
0.008	0.9936	0.9936	0.9936	0.9935	0.9936
0.010	0.9921	0.9920	0.9920	0.9921	0.9922

(d) Ovalbumin- Maltose-Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.9971	0.9971	0.9971	0.9970	0.9970
0.006	0.9958	0.9957	0.9956	0.9956	0.9956
0.008	0.9947	0.9946	0.9945	0.9945	0.9945
0.010	0.9932	0.9932	0.9931	0.9931	0.9931

(e) Ovalbumin- Buffer system (pH 8.9)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.9962	0.9966	0.9966	0.9967	0.9966
0.006	0.9945	0.9952	0.9954	0.9954	0.9948
0.008	0.9933	0.9936	0.9938	0.9938	0.9935
0.010	0.9917	0.9920	0.9922	0.9923	0.9920

(f) Ovalbumin- Maltose-Buffer System (pH 8.9)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.9971	0.9971	0.9970	0.9970	0.9971
0.006	0.9958	0.9959	0.9958	0.9958	0.9959
0.008	0.9946	0.9945	0.9945	0.9945	0.9945
0.010	0.9933	0.9932	0.9931	0.9930	0.9930

Table 2.2: Apparent Specific Volume, $(1-V_0)/c$ (ml/g), as functions of temperature and concentration for the following systems:

(a) Ovalbumin- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.7750	0.7750	0.8250	0.8250	0.8000
0.006	0.8000	0.8000	0.8000	0.8000	0.8000
0.008	0.8000	0.7875	0.8000	0.8125	0.8000
0.010	0.7900	0.7800	0.7800	0.7900	0.7900

(b) Ovalbumin- Maltose-Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.7250	0.7500	0.7500	0.7250	0.7250
0.006	0.7167	0.7333	0.7333	0.7000	0.7167
0.008	0.7125	0.7250	0.7250	0.6875	0.6875
0.010	0.7000	0.7200	0.7100	0.6800	0.6800

(c) Ovalbumin- Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.7500	0.7500	0.7750	0.8000	0.8000
0.006	0.8000	0.8000	0.7833	0.7833	0.7833
0.008	0.8000	0.8000	0.8000	0.8125	0.8000
0.010	0.7900	0.8000	0.8000	0.7900	0.7800

(d) Ovalbumin- Maltose-Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.7250	0.7250	0.7250	0.7500	0.7500
0.006	0.7000	0.7167	0.7333	0.7333	0.7333
0.008	0.6625	0.6750	0.6875	0.6875	0.6875
0.010	0.6800	0.6800	0.6900	0.6900	0.6900

(e) Ovalbumin- Buffer System (pH 8.9)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.9500	0.8500	0.8500	0.8250	0.8500
0.006	0.9167	0.8000	0.7667	0.7667	0.8667
0.008	0.8375	0.8000	0.7750	0.7750	0.8125
0.010	0.8300	0.8000	0.7800	0.7700	0.8000

(f) Ovalbumin- Maltose-Buffer System (pH 8.9)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.7250	0.7250	0.7500	0.7500	0.7250
0.006	0.7000	0.6833	0.7000	0.7000	0.6833
0.008	0.6750	0.6875	0.6875	0.6875	0.6875
0.010	0.6700	0.6800	0.6900	0.7000	0.7000

Table 2.3: $(\beta/\beta_o - V_o)/c$ as functions of temperature and concentration for the following systems:

(a) Ovalbumin- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.1898	0.1953	0.3273	0.3874	0.2359
0.006	0.2224	0.2396	0.1871	0.2757	0.1259
0.008	0.2234	0.1942	0.1939	0.2685	0.1011
0.010	0.1864	0.1168	0.1312	0.1538	0.1286

(b) Ovalbumin-Maltose- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.2134	0.2885	0.3048	0.2801	0.3060
0.006	0.1501	0.2109	0.2038	0.1752	0.2205
0.008	0.0882	0.1591	0.1314	0.1189	0.1402
0.010	0.0583	0.1279	0.0955	0.0782	0.0951

(c) Ovalbumin- Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.1776	0.1833	0.2028	0.2281	0.2969
0.006	0.2899	0.2765	0.2469	0.2456	0.2459
0.008	0.2539	0.2893	0.2576	0.3038	0.2666
0.010	0.2527	0.2474	0.2230	0.2049	0.1787

(d) Ovalbumin- Maltose- Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.1823	0.1980	0.2315	0.2819	0.3237
0.006	0.0511	0.1004	0.1735	0.1901	0.1953
0.008	-0.0309	0.0055	0.1032	0.1024	0.0627
0.010	-0.0470	-0.0077	0.0944	0.0763	0.0825

(e) ***Ovalbumin- Buffer System (pH 8.9)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.5960	0.4088	0.2782	0.2965	0.4464
0.006	0.3936	0.2515	0.1647	0.2159	0.4345
0.008	0.2151	0.2575	0.1761	0.2131	0.2744
0.010	0.1882	0.2491	0.1708	0.1674	0.2546

(f) ***Ovalbumin- Maltose- Buffer System (pH 8.9)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.1783	0.2027	0.2437	0.2691	0.2786
0.006	0.0289	0.0039	0.0831	0.1113	0.1056
0.008	-0.0800	-0.0133	0.0154	0.0317	0.0616
0.010	-0.1076	-0.0513	-0.0118	0.0180	0.0592

Table 2.4: Partial Specific Volume, $\bar{v}^0$ (ml/g), as functions of temperature and pH for the following systems:

(a) ***Ovalbumin- Buffer System***

pH of the system	Temperature K 298.15	303.15	308.15	313.15	318.15
2.4	0.7703	0.7802	0.8448	0.8371	0.8080
7.0	0.7430	0.7350	0.7575	0.7967	0.8060
8.9	1.0373	0.8650	0.8635	0.8715	0.9038

(b) ***Ovalbumin- Maltose- Buffer System***

pH of the system	Temperature K 298.15	303.15	308.15	313.15	318.15
2.4	0.7413	0.7665	0.7745	0.7498	0.7598
7.0	0.7523	0.7610	0.7617	0.7942	0.7942
8.9	0.7590	0.7598	0.7743	0.7663	0.7237

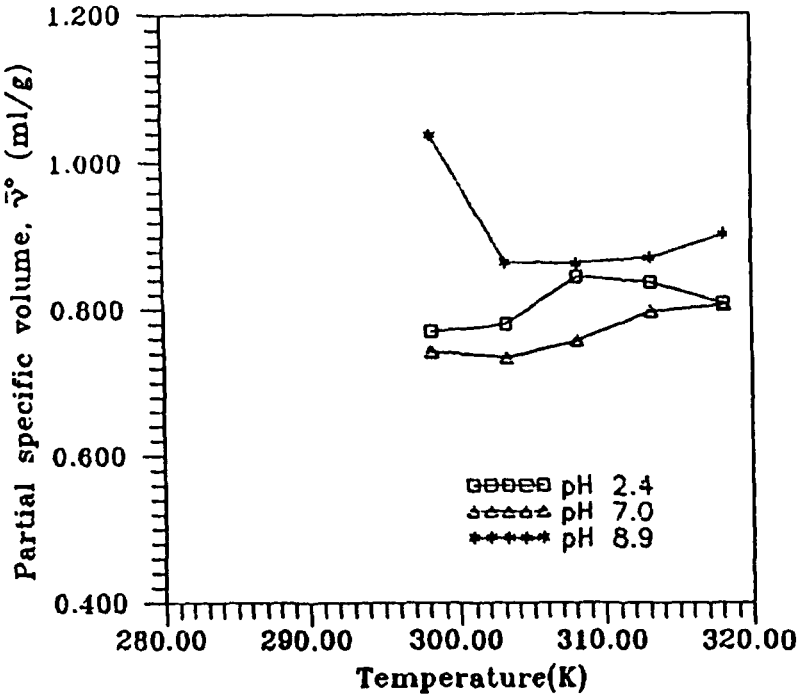

Fig 2.1(a) Plots of partial specific volume versus temperature for ovalbumin at different pH values

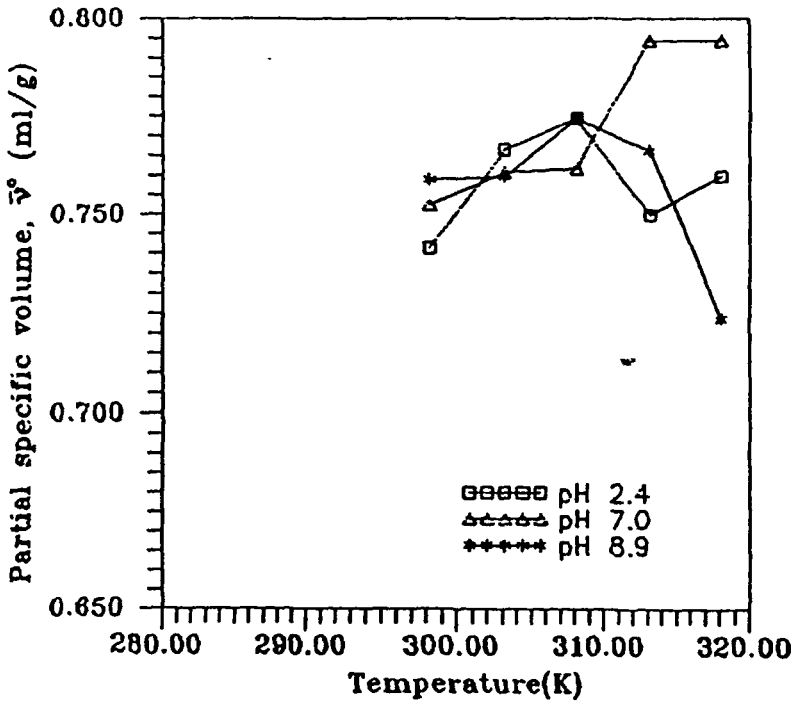

Fig 2.1(b) Plots of partial specific volume versus temperature for ovalbumin–maltose system at different pH values

Table 2.5: Partial Specific Adiabatic Compressibility (β_s x 10^{12} cm^2/dyne), as functions of temperature and pH for the following systems:

(a) Ovalbumin- Buffer System

pH of the system	Temperature K				
	298.15	303.15	308.15	313.15	318.15
2.4	11.8065	15.7412	20.8916	26.2210	14.0037
7.0	10.2139	10.2174	11.4957	13.0105	18.6215
8.9	34.2240	22.1682	14.7291	16.9894	27.5892

(b) Ovalbumin- Maltose- Buffer System

pH of the system	Temperature K				
	298.15	303.15	308.15	313.15	318.15
2.4	12.1352	14.3606	15.8636	15.0490	16.5338
7.0	11.8954	12.2924	12.1029	14.8612	16.8930
8.9	13.0261	11.6493	13.8546	14.8888	14.6808

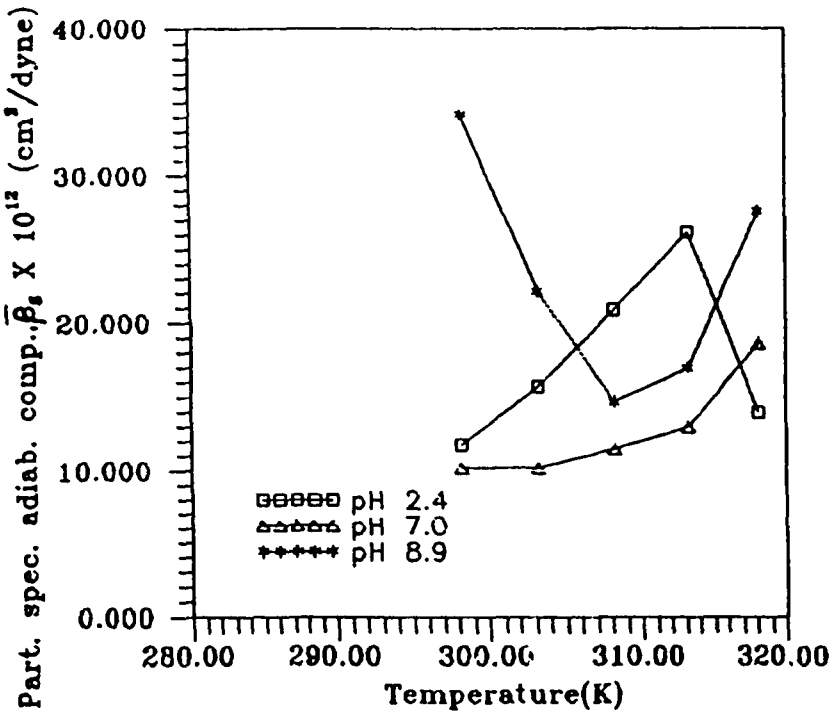

Fig 2.2(a) Plots of partial specific adiabatic compressibility versus temperature for ovalbumin at different pH values

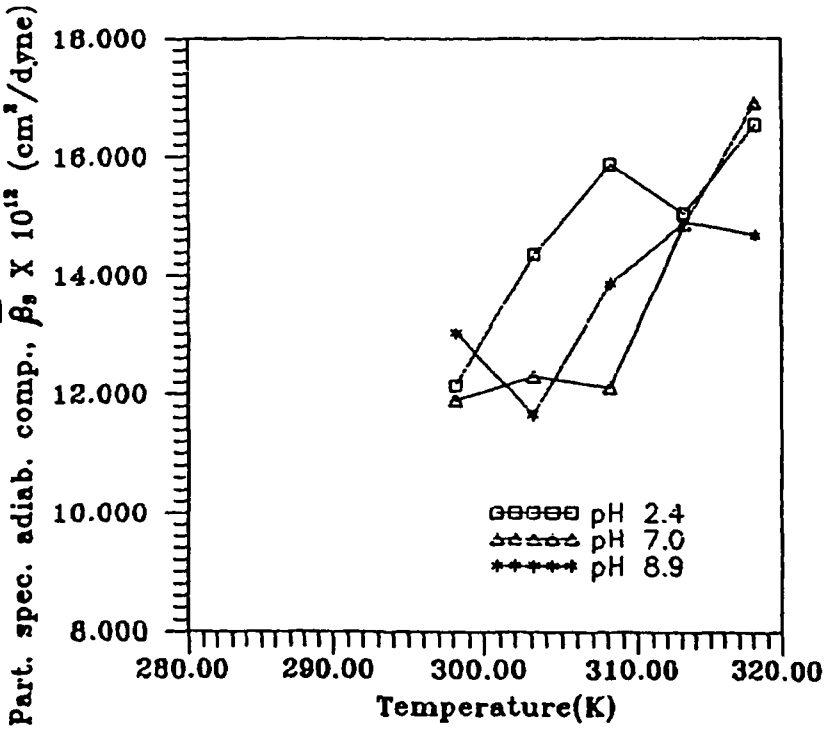

Fig 2.2(b) Plots of partial specific adiabatic compressibility versus temperature for ovalbumin–maltose system at different values

V_{cav} contributes positively and both terms have been known to tend to cancel almost completely. This makes it possible to calculate the partial specific volume of a protein as the sum of constitutive atomic or group volumes [11,145-147].

Since the constitutive atomic volume, V_c, should be approximated as incompressible, the differentiation of equation 2.5 with pressure under adiabatic conditions give

$$\bar{\beta}_s = -(1 / \bar{v}^0) \quad (\partial \bar{v}^0 / \partial P)$$

$$\bar{\beta}_s = -(1 / \bar{v}^0) [\partial V_{cav} / \partial P + \partial \Delta V_{soln} / \partial P] \qquad 2.6$$

Thus, the partial specific adiabatic compressibility obtained experimentally for different systems is mainly contributed from the cavities and solvation. The first term in equation 2.6 contributes positively while the second term contributes negatively to $\bar{\beta}_s$. As apparent from tables 2.5, the $\bar{\beta}_s$ values for all the systems studied is positive suggesting the presence of highly compressible cavities in the protein molecules and that the effect of cavity has overcome the solvation effect.

An examination of table 2.4 shows that the partial specific volume $\bar{v}^0$, of ovalbumin at pH 7.0 is less than that at pH 2.4. and 8.9. This shows the native or compact form of protein at pH 7.0 upto 40.0 °C. At pH 2.4 and 8.9 the protein is denatured and the random coils are formed, this is evident from the higher values of $\bar{v}^0$. Comparison of tables 2.4 (a) and (b) reveals that the addition of maltose to the protein solution decreases the partial specific volume of the protein and thus decreasing the compressibility of the solution. The partial specific volume of the protein is directly related to its compressibility. An increase in the value of $\bar{v}^0$ increases the value of $\bar{\beta}_s$. The decrease in the values of $\bar{v}^0$ and $\bar{\beta}_s$ after the addition of maltose may be attributed to the fact that the presence of sugar strengthen the pair-wise hydrophobic interactions between the hydrophobic groups [8] of the protein molecule thus maintaining the globular form of the protein in the solution. When the protein undergoes denaturation random coils are formed, therefore, there is an increase in the values of $\bar{v}^0$ and $\bar{\beta}_s$ at pH 2.4 and 8.9.

CHAPTER -3

ISOTHERMAL COMPRESSIBILITY AND INTERNAL PRESSURE

INTRODUCTION

Among the various thermodynamic parameters that have been derived from the ultrasonic velocity measurement , the internal pressure and the isothermal compressibility are known to be the key parameters in understanding the nature of molecular interactions in liquids.

The internal pressure is a fundamental property of the liquid state which helps in exploring the nature of intermolecular interactions in solutions. Dunlop et al [148] determined the internal pressure of different liquid mixtures and compared it with their cohesive energy density values. Stavely et al [149] predicted the interactions in liquid mixtures by comparing the internal pressure of individual components. Buchler and coworkers proposed another relation [150], which has been used for molten salts and liquid metals [97,151,152]. Isothermal compressibility has been widely evaluated by many workers for pure liquids [35,36], liquid mixture [80,153] and electrolytes.

The Pseudo-Grüneisen parameter, Γ, is a useful parameter for the study of thermodynamics of any system, and has been calculated by several workers [99,155].

Hildebrand and Scott introduced a parameter known as solubility parameter in the theory of solutions. It is the square root of the cohesive energy density. The importance of this parameter has been demonstrated by a number of workers [95,96].

Surface tension is an important phenomenon in the study of molecular chemistry. Its measurements find valuable applications in the biological sciences, particularly in bacteriology; the movement of moisture in the soil and the passage of sap in plants are only two of the many agricultural phenomena that involve surface tension. Various attempts have been made for theoretical evaluation of surface tension of liquids, molten salts, [31,156-163], but only few attempts [164] have been made for aminoacid solutions. So in the present work we have tried to

evaluate the surface tension of aminoacids and protein solution using the equation given by McGowan.

Consequently, we have evaluated the isothermal compressibility, the internal pressure, the solubility parameter, the Pseudo-Grüneisen parameter and surface tension from the ultrasonic velocity and density measurements.

THEORY:

The following relation for isothermal compressibility has been given by McGowan [79],

$$\beta_T \sigma^{3/2} = 1.33 \times 10^{-8} \qquad 3.1$$

where σ is the surface tension and β_T is the isothermal compressibility of the solutions. The Auerbach relationship [163] between the speed of sound (U) and the surface tension σ is given by

$$U = [\sigma / (6.4 \times 10^{-4} \rho)]^{2/3} \qquad 3.2$$

Thus from equations 3.1 and 3.2 we have

$$\beta_T = 1.33 \times 10^{-8} / (6.4 \times 10^{-4} U^{3/2} \rho)^{3/2} \qquad 3.3$$

This relation has been employed by a number of workers during recent years [92,156,157,165,166] in case of pure liquids, liquid mixtures, non-electrolytes, molten salts and liquid metals. Another relation for isothermal compressibility has been proposed by J.D. Pandey [82], which is expressed as

$$\beta_T = (17.1 \times 10^{-4}) / (T^{4/9} U^2 \rho^{4/3}) \qquad 3.4$$

This relation has also been employed by several workers for aminoacids and carbohydrates [68,167].

Internal pressure, P_i, is expressed as

$$P_i = \alpha T / \beta_T \qquad 3.5$$

where β_T is the isothermal compressibility obtained from equation 3.3 and α is the thermal expansion coefficient.

$$\alpha = -1/\rho \, (d\rho / dT)_P \qquad 3.6$$

The plots of ρ vs T give the value of $(d\rho / dT)_P$.

The solubility parameter, δ , which is the square-root of cohesive energy density, can be calculated using the relation given by Hildebrand and Scott [84] i.e.,

$$\delta = (\alpha T / \beta_T)^{1/2} \qquad 3.7$$

The Pseudo-Grüneisen parameter, Γ, is calculated by using the expression

$$\Gamma = \gamma - 1 / \alpha T \qquad 3.8$$

where γ is the specific heat ratio. It may be calculated by using the relation [153],

$$\beta_T = \beta_s \gamma \qquad 3.9$$

RESULTS AND DISCUSSION

The isothermal compressibility β_T for ovalbumin-buffer systems, ovalbumin-maltose-buffer systems and amino acid-urea-water systems have been computed using equations 3.3 and 3.4 both. The values of β_T obtained for these systems are presented in tables 3.1 a-h. A fairly good agreement has been found between the β_T values obtained from these two relations. The plots of β_T as functions of concentration and temperature [3.1 a-h] show an inverse relationship of β_T with temperature as well as the concentration of solutes indicating that the presence of incompressible ions or molecules increase with an increase in the concentration of the solution. In case of ovalbumin-maltose-buffer system, there is a marked decrease in β_T indicating that the compressible nature of protein in

Table 3.1: Isothermal Compressibility, (β_T x 10^{10},m^2N^{-1}), as functions of temperature and concentration for the following systems:

(a) Ovalbumin- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	5.7227	5.6584	5.5978	5.5408	5.5090
	5.9086	*5.8065*	*5.7096*	*5.6175*	*5.5496*
0.004	5.7056	5.6416	5.5838	5.5286	5.4936
	5.8930	*5.7897*	*5.6968*	*5.6065*	*5.5330*
0.006	5.6980	5.6349	5.5722	5.5188	5.4815
	5.8821	*5.7851*	*5.6863*	*5.5976*	*5.5250*
0.008	5.6895	5.6241	5.5640	5.5106	5.4711
	5.8782	*5.7752*	*5.6788*	*5.5903*	*5.5156*
0.010	5.6794	5.6116	5.5525	5.4969	5.4638
	5.8689	*5.7638*	*5.6684*	*5.5779*	*5.5091*

(b) Ovalbumin- Maltose-Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	3.3648	3.3508	3.3428	3.3379	3.3369
	3.6833	*3.6446*	*3.6106*	*3.5801*	*3.5541*
0.004	3.3565	3.3438	3.3359	3.3310	3.3300
	3.6772	*3.6378*	*3.6039*	*3.5735*	*3.5475*
0.006	3.3513	3.3381	3.3302	3.3249	3.3426
	3.6721	*3.6324*	*3.5985*	*3.5678*	*3.5426*
0.008	3.3441	3.3325	3.3242	3.3193	3.3188
	3.6657	*3.6269*	*3.5926*	*3.5624*	*3.5369*
0.010	3.3382	3.3269	3.3182	3.3133	3.3128
	3.6594	*3.6215*	*3.5869*	*3.5567*	*3.5313*

(c) Ovalbumin- Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	5.5999	5.5350	5.4786	5.4216	5.3845
	5.7958	*5.6938*	*5.6013*	*5.5099*	*5.4379*
0.004	5.5833	5.5187	5.4633	5.4073	5.3718
	5.7805	*5.6789*	*5.5867*	*5.4956*	*5.4252*
0.006	5.5784	5.5130	5.4560	5.3994	5.3624
	5.7760	*5.6737*	*5.5808*	*5.4898*	*5.4181*
0.008	5.5693	5.5065	5.4488	5.3938	5.3553
	5.7676	*5.6677*	*5.5743*	*5.4848*	*5.4118*
0.010	5.5619	5.4968	5.4392	5.3820	5.3436
	5.7608	*5.6588*	*5.5655*	*5.4741*	*5.4012*

(d) Ovalbumin- Maltose-Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	3.3917	3.3827	3.3773	3.3745	3.3731
	3.7115	*3.6754*	*3.6436*	*3.6150*	*3.5883*
0.004	3.3829	3.3748	3.3693	3.3670	3.3660
	3.7029	*3.6674*	*3.6360*	*3.6079*	*3.5812*
0.006	3.3758	3.3677	3.3636	3.3613	3.3586
	3.6960	*3.6610*	*3.6305*	*3.6025*	*3.5750*
0.008	3.3688	3.3608	3.3580	3.3553	3.3525
	3.6892	*3.6542*	*3.6251*	*3.5967*	*3.5689*
0.010	3.3618	3.3546	3.3528	3.3492	3.3478
	3.6824	*3.6483*	*3.6201*	*3.5909*	*3.5643*

(e) ***Ovalbumin-Buffer System (pH 8.9)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	5.5316	5.4685	5.4130	5.3616	5.3257
	5.7329	*5.6330*	*5.5417*	*5.4557*	*5.3852*
0.004	5.5226	5.4565	5.4003	5.3498	5.3148
	5.7246	*5.6219*	*5.5272*	*5.4422*	*5.3754*
0.006	5.5112	5.4460	5.3884	5.3389	5.3086
	5.7141	*5.6124*	*5.5193*	*5.4351*	*5.3698*
0.008	5.4982	5.4388	5.3805	5.3311	5.2970
	5.7021	*5.6058*	*5.5121*	*5.4281*	*5.3594*
0.010	5.4885	5.4309	5.3719	5.3210	5.2893
	5.6932	*5.5985*	*5.5042*	*5.4189*	*5.3524*

(f) ***Ovalbumin- Maltose-Buffer System (pH 8.9)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	3.3617	3.3512	3.3428	3.3388	3.3374
	3.6823	*3.6450*	*3.6106*	*3.5810*	*3.5545*
0.004	3.3529	3.3429	3.3350	3.3310	3.3301
	3.6738	*3.6370*	*3.6030*	*3.5736*	*3.5476*
0.006	3.3460	3.3355	3.3281	3.3246	3.3232
	3.6670	*3.6299*	*3.5964*	*3.5674*	*3.5411*
0.008	3.3373	3.3286	3.3212	3.3177	3.3171
	3.6585	*3.6231*	*3.5898*	*3.5608*	*3.5354*
0.010	3.3304	3.3217	3.3147	3.3116	3.3115
	3.6517	*3.6164*	*3.5836*	*3.5551*	*3.5301*

(g) ***L-Valine-Urea-Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.0000	5.8533	5.7831	5.7227	5.6678	5.6267
	6.0283	***5.9201***	***5.8226***	***5.7317***	***5.6549***
0.0502	5.7854	5.7297	5.6650	5.6156	5.5686
	5.9661	***5.8715***	***5.7704***	***5.6848***	***5.6029***
0.1008	5.7279	5.6806	5.6199	5.5694	5.5254
	5.9134	***5.8267***	***5.7296***	***5.6432***	***5.5643***
0.1520	5.6778	5.6328	5.5778	5.5285	5.4897
	5.8674	***5.7831***	***5.6914***	***5.6064***	***5.5323***
0.2035	5.6175	5.5789	5.5246	5.4737	5.4323
	5.8120	***5.7339***	***5.6431***	***5.5569***	***5.4809***
0.2555	5.5613	5.5249	5.4713	5.4195	5.3779
	5.7603	***5.6845***	***5.5947***	***5.5080***	***5.4321***
0.3081	5.5189	5.4756	5.4250	5.3785	5.3450
	5.7212	***5.6394***	***5.5526***	***5.4710***	***5.4025***
0.3610	5.4743	5.4236	5.3745	5.3309	5.3032
	5.6801	***5.5918***	***5.5066***	***5.4279***	***5.3649***
0.4144	5.4335	5.3715	5.3239	5.2861	5.2648
	5.6424	***5.5441***	***5.4605***	***5.3874***	***5.3304***

(h) ***L-Serine-Urea- Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.1001	5.7724	5.6930	5.6154	5.5557	5.4151
	5.9542	***5.8381***	***5.7255***	***5.6309***	***5.5542***
0.2002	5.6583	5.5909	5.5312	5.4823	5.4454
	5.8495	***5.7449***	***5.6491***	***5.5648***	***5.4926***
0.3004	5.5578	5.4983	5.4511	5.4101	5.3745
	5.7570	***5.7356***	***5.5763***	***5.4996***	***5.4290***
0.4005	5.4691	5.4132	5.3740	5.3392	5.3049
	5.6753	***5.5823***	***5.5062***	***5.4354***	***5.3665***
0.5006	5.3262	5.3337	5.2990	5.2671	5.2356
	5.5987	***5.5094***	***5.4378***	***5.3701***	***5.3042***
0.6007	5.3079	5.2580	5.2269	5.1978	5.1683
	5.5264	***5.4398***	***5.3720***	***5.3073***	***5.2435***
0.7009	5.2425	5.1911	5.1583	5.1296	5.0999
	5.4662	***5.3782***	***5.3093***	***5.2454***	***5.1818***
0.8010	5.1767	5.1245	5.0901	5.0604	5.0319
	5.4047	***5.3169***	***5.2468***	***5.1824***	***5.1203***
0.9014	5.1230	5.0671	5.0251	4.9917	4.9651
	5.3549	***5.2639***	***5.1873***	***5.1198***	***5.0598***
1.0012	5.0743	5.0133	4.9627	4.9241	4.8980
	5.3097	***5.2142***	***5.1299***	***5.0582***	***4.9990***

(The values given in bold have been calculated by Pandey's relation)

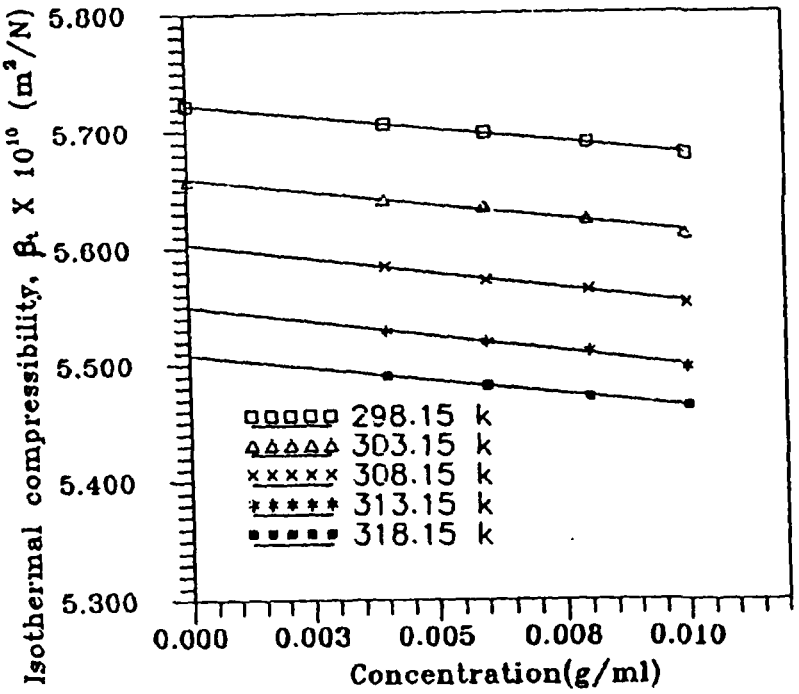

Fig 3.1(a) Plots of isothermal compressibility versus concentration for ovalbumin at pH 2.4

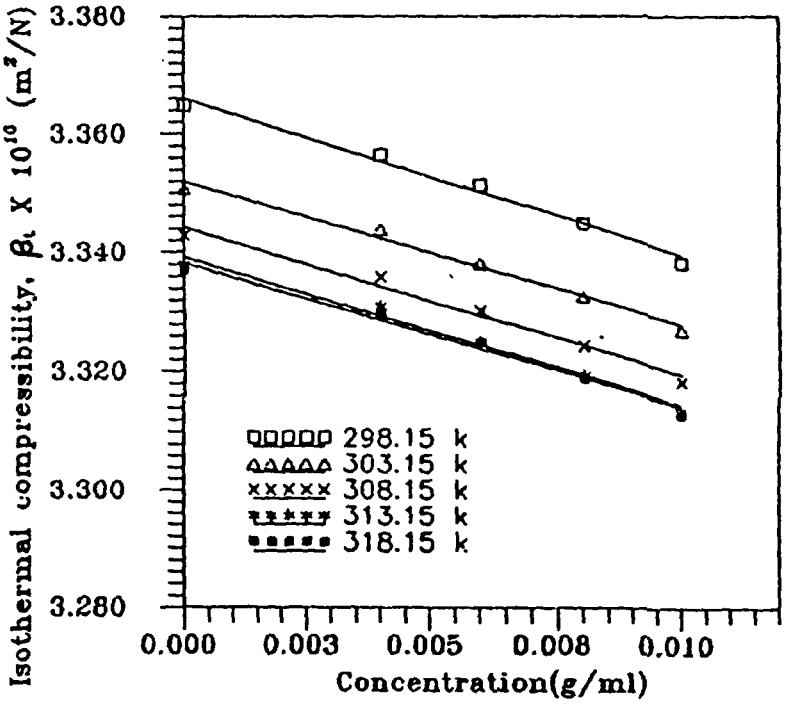

Fig 3.1(b) Plots of isothermal compressibility versus concentration for ovalbumin–maltose system at pH 2.4

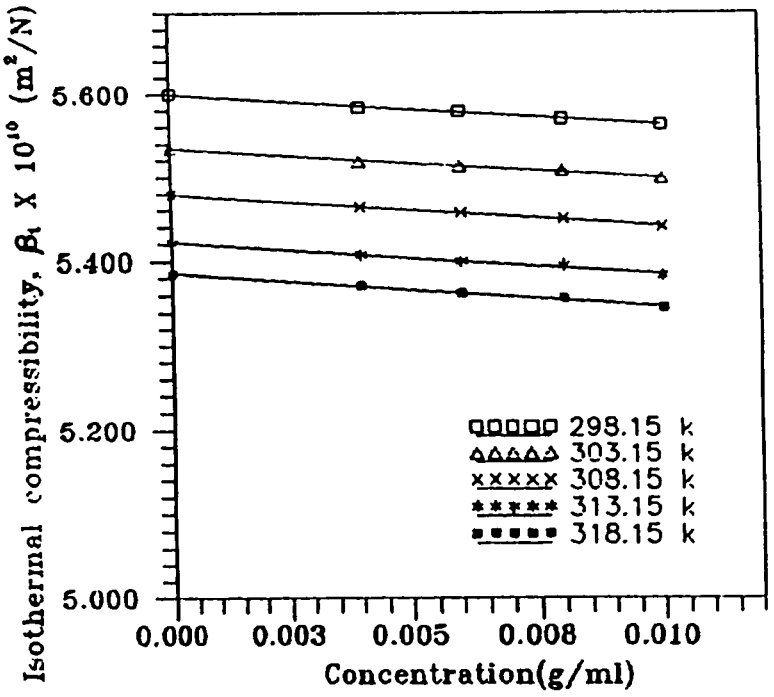

Fig 3.1(c) Plots of isothermal compressibility versus concentration for ovalbumin at pH 7.0

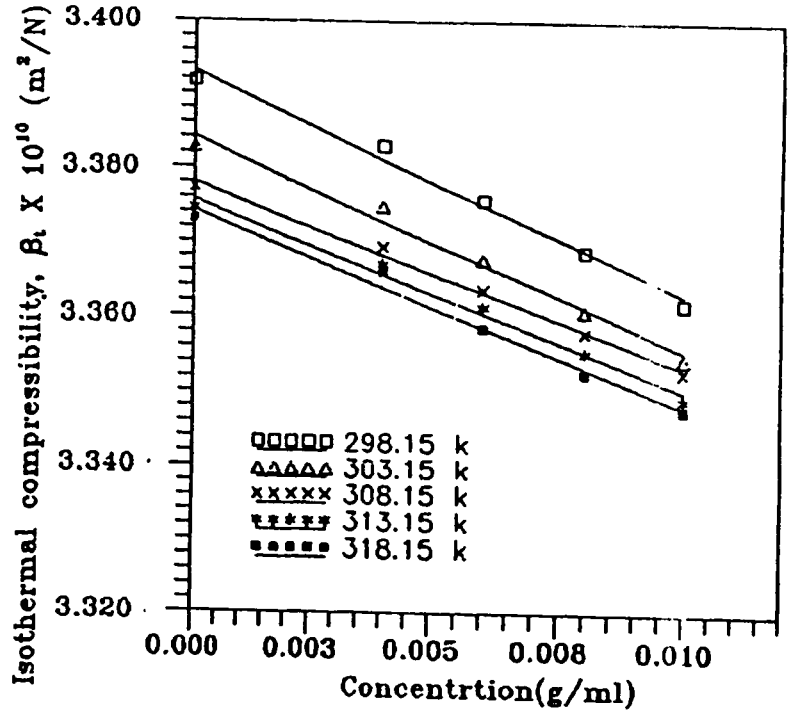

Fig 3.1(d) Plots of isothermal compressibility versus concentration for ovalbumin–maltose system at pH 7.0

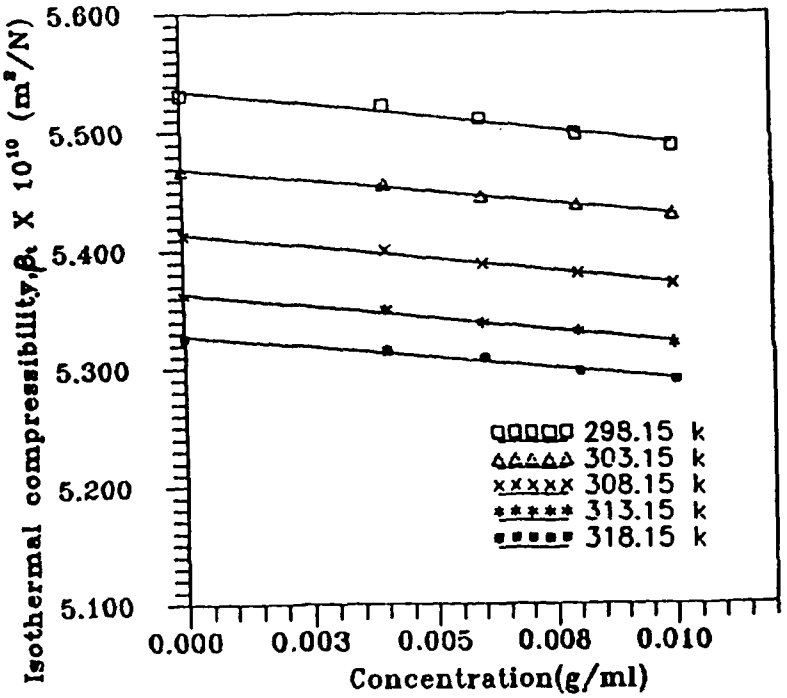

Fig 3.1(e) Plots of isothermal compressibility versus concentration for ovalbumin at pH 8.9

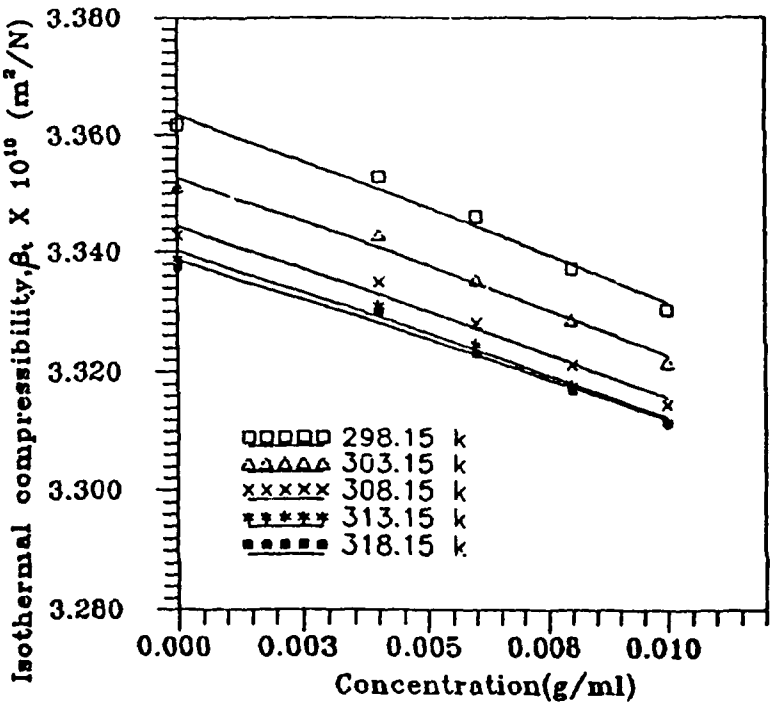

Fig 3.1(f) Plots of isothermal compressibility versus concentration for ovalbumin–maltose system at pH 8.9

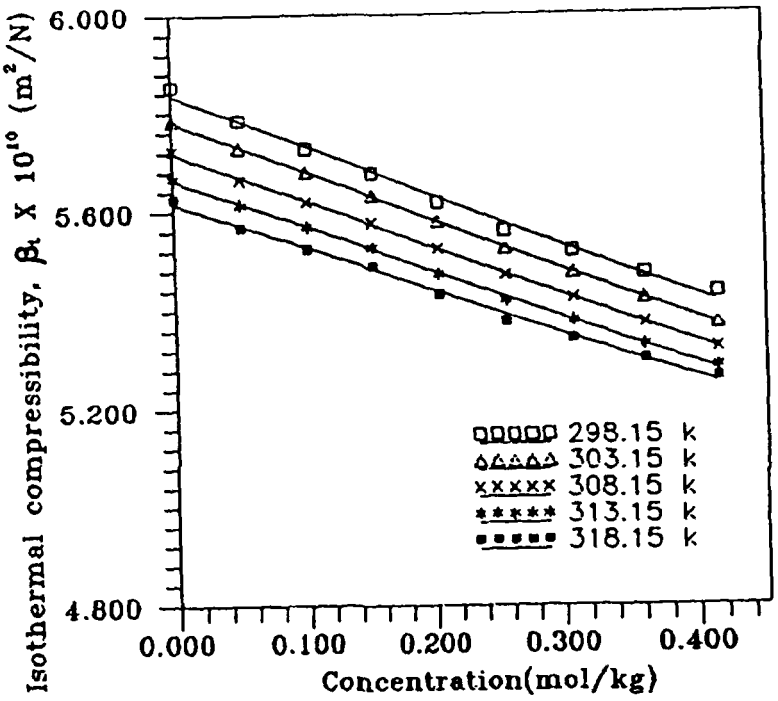

Fig 3.1(g) Plots of isothermal compressibility versus concentration for L-valine-urea-water system

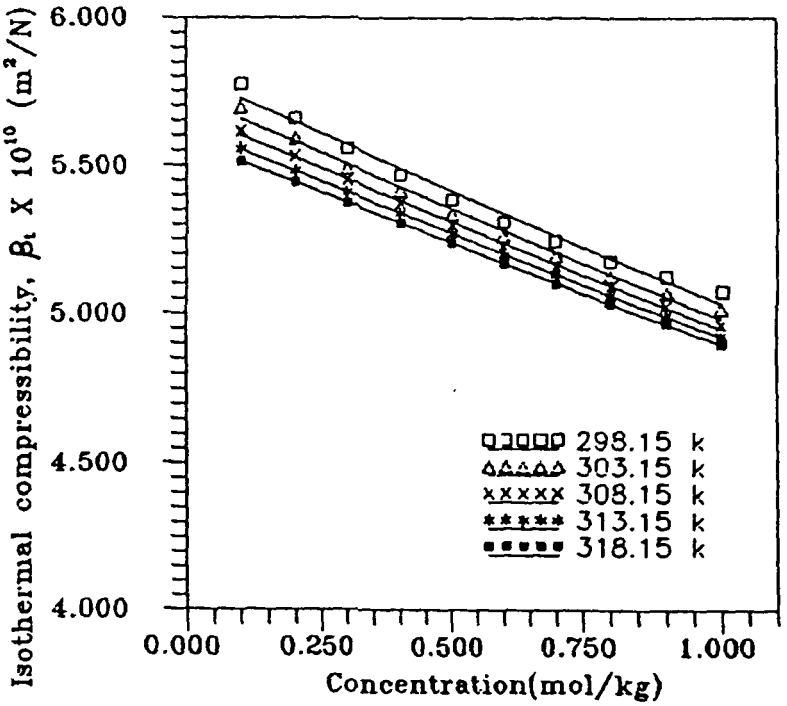

Fig 3.1(h) Plots of isothermal compressibility versus concentration for L-serine-urea-water system

solution decreases in the presence of sugar and the extent of decrease in compressibility is different at different pH values. The variation of β_T with temperature and concentration follow the trend similar to that of β_s but the values of β_T are slightly higher than those of β_s.

The computed values of internal pressure are listed in the tables 3.2 a-h. An examination of these tables reveals that as the temperature increases the values of P_i increase. This increase in the values of P_i seems to be associated with

(i) an increase in the repulsive forces and decrease in the attractive forces among the molecules of the solution.

(ii) an increase in the kinetic energy of the system with temperature.

The addition of maltose to ovalbumin-buffer system increases the internal pressure to a greater extent. Sugars strengthen different types of interactions occurring in the molecules of protein in solutions. This strengthening of interactions (or stabilization of protein) decreases the isothermal compressibility of the solution, which in turn increases the internal pressure of the system. Internal pressure also varies with composition but no regular trend is observed in this case.

The values of solubility parameter, δ, obtained as the square root of internal pressure, are recorded in tables 3.3 a-h. The values of δ increase with increase in temperature. Such an increase may be attributed to an increase in the cohesive energy density which is the energy of isothermal vaporization from the liquid to the ideal state per unit volume of the liquid. The values of δ show an irregular trend with composition.

The values of Pseudo-Grüneisen parameter, Γ, obtained by using equation 3.8 are listed in tables 3.4 a-h. The values of Γ are found to decrease with the increase in temperature while an irregular trend is observed with the concentration of the solution.

Table 3.2: Internal Pressure ($P_i x 10^{-8} Nm^{-2}$), as functions of temperature and concentration for the following systems:

(a) Ovalbumin- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	0.7141	1.2678	1.8525	2.4693	3.1071
0.004	0.8757	1.3741	1.8998	2.4537	3.0273
0.006	0.7163	1.2714	1.8587	2.4760	3.1187
0.008	0.7379	1.2947	1.8828	2.5017	3.1468
0.010	0.6392	1.2402	1.8744	2.5436	3.2372

(b) Ovalbumin-Maltose- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	1.3797	1.9482	2.5372	3.1479	3.7768
0.004	1.5513	2.3397	3.1577	4.0051	4.8798
0.006	1.5534	2.3432	3.1623	4.0115	4.8862
0.008	1.7230	2.4092	3.1209	3.8572	4.6158
0.010	1.6388	2.3453	3.0800	3.8377	4.6198

(c) Ovalbumin - Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	0.9854	1.4021	1.8403	2.3030	2.7811
0.004	1.0506	1.4700	1.9108	2.3761	2.8561
0.006	0.7663	1.2863	1.8344	2.4131	3.0132
0.008	1.0286	1.4165	1.8252	2.2554	2.7007
0.010	1.1489	1.4636	1.7941	2.1423	2.5006

(d) Ovalbumin – Maltose - Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	2.6698	3.0679	3.4779	3.8997	4.3342
0.004	2.6231	3.0845	3.5611	4.0517	4.5572
0.006	2.7939	3.1525	3.5204	3.8990	4.2910
0.008	2.9389	3.2366	3.5397	3.8527	4.1756
0.010	2.9167	3.2352	3.5594	3.8964	4.2420

(e) Ovalbumin - Buffer System (pH 8.9)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	1.1573	1.5044	1.8689	2.2516	2.6464
0.004	0.7072	1.2139	1.7481	2.3097	2.8930
0.006	0.2211	0.9649	1.7509	2.5783	3.4394
0.008	0.7112	1.2341	1.7865	2.3670	2.9698
0.010	0.6709	1.2087	1.7774	2.3753	2.9952

(f) Ovalbumin -Maltose- Buffer System (pH 8.9)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	1.5157	2.2580	3.0292	3.8266	4.6508
0.004	1.6607	2.3425	3.0507	3.7821	4.5380
0.006	1.5491	2.2731	3.0247	3.8017	4.6048
0.008	1.6395	2.3443	3.0763	3.8330	4.6142
0.010	1.9014	2.5262	3.1744	3.8438	4.5335

(g) L- Valine-Urea-Water System

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.0000	1.5867	1.8010	2.0232	2.2556	2.4918
0.0502	1.6116	1.8234	2.0491	2.2803	2.5218
0.1008	1.5407	1.7797	2.0354	2.3001	2.5752
0.1520	1.5291	1.7536	1.9926	2.2411	2.4971
0.2035	1.6111	1.8220	2.0483	2.2844	2.5276
0.2555	1.6390	1.8672	2.1124	2.3693	2.6337
0.3081	1.6511	1.8832	2.1293	2.3859	2.6482
0.3610	1.6985	1.9202	2.1521	2.3928	2.6370
0.4144	1.7145	1.9579	2.2084	2.4666	2.7280

(h) L- Serine-Urea-Water System

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.1001	0.5048	1.0210	1.5695	2.1459	2.7459
0.2002	0.8920	1.1360	1.8626	2.3844	2.9275
0.3004	0.9525	1.4085	1.9007	2.4085	2.9390
0.4005	0.9214	1.3880	1.8751	2.3846	2.9181
0.5006	0.8213	1.3213	1.8426	2.3879	2.9591
0.6007	1.0275	1.5059	2.0030	2.5225	3.0667
0.7009	0.8444	1.3708	1.9192	2.4923	3.0927
0.8010	1.1616	1.6200	2.0967	2.5941	3.1138
0.9014	0.8813	1.4345	2.0142	2.6196	3.2503
1.0012	0.7334	1.3368	1.9723	2.6371	3.3276

Table 3.3: Solubility parameter [δ x 10^{-4}, $(Nm^{-2})^{1/2}$] as functions of temperature and concentration for the following systems:

(a) Ovalbumin- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	0.8450	1.1259	1.3611	1.5714	1.7627
0.004	0.9358	1.1722	1.3784	1.5665	1.7404
0.006	0.8463	1.1276	1.3634	1.5736	1.7660
0.008	0.8590	1.1379	1.3721	1.5817	1.7739
0.010	0.7994	1.1136	1.3691	1.5949	1.7992

(b) Ovalbumin- Maltose- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	1.1746	1.3958	1.5929	1.7742	1.9434
0.004	1.2455	1.5296	1.7770	2.0013	2.2090
0.006	1.2464	1.5308	1.7782	2.0029	2.2105
0.008	1.3126	1.5522	1.7666	1.9640	2.1484
0.010	1.2802	1.5314	1.7550	1.1959	2.1494

(c) Ovalbumin- Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	0.9927	1.1841	1.3566	1.5176	1.6677
0.004	0.9358	1.2124	1.3823	1.5415	1.6900
0.006	0.8754	1.1342	1.3544	1.5534	1.7358
0.008	1.0141	1.1902	1.3510	1.5018	1.6434
0.010	1.0718	1.2098	1.3394	1.4637	1.5813

(d) Ovalbumin- Maltose- Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	1.6340	1.7515	1.8649	1.9748	2.0819
0.004	1.6196	1.7563	1.8871	2.0129	2.1348
0.006	1.6715	1.7755	1.8763	1.9746	2.0715
0.008	1.7143	1.7990	1.8814	1.9628	2.0434
0.010	1.7078	1.7987	1.8866	1.9739	2.0599

(e) Ovalbumin- Buffer System (pH 8.9)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	1.0758	1.2265	1.3671	1.5005	1.6268
0.004	0.8410	1.1018	1.3222	1.5198	1.7009
0.006	0.4702	0.9823	1.3232	1.6057	1.8546
0.008	0.8433	1.1109	1.3366	1.5385	1.7233
0.010	0.8191	1.0994	1.3332	1.5412	1.7307

(f) Ovalbumin- Maltose- Buffer System (pH 8.9)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	1.2311	1.5027	1.7405	1.9562	2.1566
0.004	1.2887	1.5305	1.7466	1.9448	2.1303
0.006	1.2446	1.5077	1.7392	1.9498	2.1459
0.008	1.2804	1.5311	1.7539	1.9578	2.1481
0.010	1.3789	1.5894	1.7817	1.9606	2.1292

(g) L- Valine- Urea- Water System

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.0000	1.2596	1.3420	1.4224	1.5019	1.5785
0.0502	1.2695	1.3503	1.4315	1.5101	1.5880
0.1008	1.2412	1.3341	1.4267	1.5166	1.6047
0.1520	1.2366	1.3242	1.4116	1.4970	1.5802
0.2035	1.2693	1.3498	1.4312	1.5119	1.5898
0.2555	1.2802	1.3665	1.4534	1.5393	1.6229
0.3081	1.2850	1.3723	1.4592	1.5446	1.6273
0.3610	1.3033	1.3857	1.4670	1.5469	1.6239
0.4144	1.3094	1.3992	1.4861	1.5705	1.6517

(h) L- Serine- Urea- Water System

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.1001	0.7105	1.0104	1.2528	1.4649	1.6571
0.2002	0.9444	1.1682	1.3647	1.5442	1.7110
0.3004	0.9760	1.1868	1.3786	1.5519	1.7144
0.4005	0.9599	1.1781	1.3694	1.5442	1.7082
0.5006	0.9063	1.1495	1.3574	1.5453	1.7202
0.6007	1.0137	1.2272	1.4153	1.5882	1.7512
0.7009	0.9189	1.1708	1.3854	1.5787	1.7586
0.8010	1.0778	1.2728	1.4480	1.6106	1.7646
0.9014	0.9388	1.1977	1.4192	1.6185	1.8029
1.0012	0.8564	1.1562	1.4044	1.6239	1.8242

Table 3.4: Pseudo - Grüineisen parameter, Γ, as functions of temperature and concentration for the following systems:

(a) Ovalbumin- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	7.6673	4.3509	3.0007	2.2694	1.8155
0.004	6.2547	4.0150	2.9269	2.2843	1.8635
0.006	7.6490	4.3717	2.9928	2.2642	1.8104
0.008	7.4267	4.2643	2.9554	2.2419	1.7952
0.010	8.5762	4.4540	2.9693	2.2060	1.7450

(b) Ovalbumin- Maltose- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	3.0957	2.1187	1.6190	1.3045	1.0895
0.004	3.1994	2.1294	1.5835	1.2549	1.0349
0.006	3.1957	2.1252	1.5810	1.2521	1.0341
0.008	2.8790	2.0663	1.6015	1.3018	1.0936
0.010	3.0989	2.1217	1.6221	1.3077	1.0921

(c) Ovalbumin- Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	5.5870	3.9567	3.0373	2.4465	2.0398
0.004	5.2407	3.7742	2.9271	2.3739	1.9880
0.006	7.1873	4.3145	3.0481	2.3358	1.8832
0.008	5.3565	3.9189	3.0648	2.4997	2.1017
0.010	4.7957	3.7941	3.1190	2.6328	2.2705

(d) ***Ovalbumin- Maltose- Buffer System (pH 7.0)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	1.8622	1.6276	1.4424	1.2924	1.1689
0.004	1.8947	1.6201	1.4082	1.2437	1.1117
0.006	1.7780	1.5828	1.4239	1.2919	1.1799
0.008	1.6891	1.5406	1.4151	1.3065	1.2115
0.010	1.7012	1.5409	1.4069	1.2910	1.1920

(e) ***Ovalbumin- Buffer System (pH 8.9)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	4.7692	3.6968	2.9982	2.5078	2.1476
0.004	7.8094	4.5831	3.2120	2.4492	1.9646
0.006	4.9927	5.7678	3.2020	2.1906	1.6531
0.008	7.7719	4.5102	3.1388	2.3870	1.9148
0.010	8.2419	4.6058	3.1557	2.3792	1.8989

(f) ***Ovalbumin-Maltose Buffer System (pH 8.9)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	3.2770	2.2066	1.6510	1.3129	1.0862
0.004	2.9897	2.1265	1.6396	1.3274	1.1125
0.006	3.2029	2.1896	1.6526	1.3205	1.1075
0.008	3.0253	2.1227	1.6238	1.3092	1.0933
0.010	2.6083	1.9690	1.5733	1.3055	1.1125

(g) ***L- Valine- Urea- Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.0000	3.4468	3.0642	2.7496	2.4870	2.2657
0.0502	3.3812	3.0124	2.7048	2.4491	2.2319
0.1008	3.5369	3.0850	2.7281	2.4281	2.1856
0.1520	3.6206	3.1762	2.8162	2.5216	2.2779
0.2035	3.4170	3.0407	2.7268	2.4643	2.2433
0.2555	3.3955	2.9971	2.6692	2.3983	2.1725
0.3081	3.3810	2.9825	2.6568	2.3882	2.1648
0.3610	3.2837	2.9273	2.6295	2.3815	2.1737
0.4144	3.2664	2.8839	2.5747	2.3201	2.1085

(h) ***L- Serine- Urea- Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.1001	10.829	5.3995	3.5442	2.6133	2.0565
0.2002	6.1517	4.0530	2.9921	2.3546	1.9309
0.3004	5.7809	3.9620	2.9374	2.3334	1.9251
0.4005	5.9937	4.0056	2.9826	2.3597	1.9408
0.5006	6.7390	4.2162	3.0396	2.3588	1.9157
0.6007	5.3942	3.7047	2.7998	2.2356	1.8506
0.7009	6.5723	4.0746	2.9257	2.2654	1.8370
0.8010	4.7780	3.4493	2.6804	2.1791	1.8266
0.9014	6.2988	3.8963	2.7926	2.1603	1.7519
1.0012	7.5644	4.1806	2.8535	2.1484	1.7131

The plots of surface tension as functions of concentration and temperature are illustrated in figures 3.2 a-h. These plots show an increase in the surface tension σ with an increase in concentration of the solution while it decreases with an increase in temperature. As is well known, surface tension of any liquid is a direct consequence of its cohesive forces, consequently, an increase in temperature effects these forces, which in turn, results in slight lowering of surface tension. Since the molecules at the surface of the solution are subject to the strong attractive forces of the interior molecules, the increase in concentration increases these cohesive forces which results in an increase in surface tension with concentration.

In case of amino acids valine and serine, valine has a non-polar side chain which induces structure in the water molecules and serine having a polar side chain undergoes hydrogen bonding with water molecules. Though they have different structures and different types of interactions with solvent molecules, there is a slight difference in the values of surface tension for valine and serine. This shows that the structure of amino acid does not effect much on the magnitude of surface tension. In case of protein, again, the addition of maltose increases the surface tension to a greater extent. This is due to the increased concentration of the solution.

Table 3.5: Surface Tension (σ x 10^3, Nm^{-1}) as functions of temperature and concentration for the following systems:

(a) Ovalbumin- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	37.8009	38.0865	38.3608	38.6235	38.7720
0.004	37.8761	38.1620	38.4253	38.6806	38.8596
0.006	37.9099	38.1922	38.4784	38.7264	38.9016
0.008	37.9475	38.2414	38.5163	38.7644	38.9511
0.010	37.9927	38.2981	38.5695	38.8292	38.9857

(b) Ovalbumin- Maltose- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	53.8591	54.0098	54.0952	54.1488	54.1593
0.004	53.9482	54.0849	54.1703	54.2232	54.2340
0.006	54.0045	54.1460	54.2315	54.2891	54.2904
0.008	54.0749	54.2072	54.2974	54.3501	54.3558
0.010	54.1453	54.2682	54.3629	54.4156	54.4214

(c) Ovalbumin- Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	38.3515	38.6505	38.9154	39.1875	39.3677
0.004	38.4273	38.7268	38.9881	39.2567	39.4295
0.006	38.4501	38.7534	39.0226	39.2953	39.4758
0.008	38.4918	38.7839	39.0570	39.3221	39.5104
0.010	38.5260	38.8297	39.1029	39.3797	39.5682

(d) ***Ovalbumin-Maltose- Buffer System (pH 7.0)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	53.5739	53.6691	53.7269	53.7562	53.7714
0.004	53.6674	53.7579	53.8109	53.8358	53.8509
0.006	53.7422	53.8284	53.8718	53.8964	53.9258
0.008	53.8164	53.9025	53.9321	53.9614	53.9906
0.010	53.8913	53.9683	53.9881	54.0269	54.0421

(e) ***Ovalbumin- Buffer System (pH 8.9)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	38.6666	38.9631	39.2291	39.4794	39.6567
0.004	38.7085	39.0205	39.2906	39.5374	39.7109
0.006	38.7618	39.0703	39.3483	39.5915	39.7417
0.008	38.8228	39.1048	39.3868	39.6301	39.7999
0.010	38.8686	39.1431	39.4291	39.6803	39.8387

(f) ***Ovalbumin-Maltose- Buffer System (pH 8.9)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	53.8924	54.0053	54.0952	54.1384	54.1537
0.004	53.9863	54.0944	54.1798	54.2230	54.2334
0.006	54.0611	54.1739	54.2546	54.2930	54.3081
0.008	54.1551	54.2493	54.3298	54.3683	54.3740
0.010	54.2301	54.3246	54.4006	54.4345	54.4352

(g) ***L- Valine- Urea- Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.0000	37.2363	37.5372	37.8007	38.0451	38.2293
0.0502	37.5273	37.7698	38.0569	38.2801	38.4950
0.1008	37.7781	37.9875	38.2603	38.4914	38.6955
0.1520	37.9997	38.2020	38.4528	38.6808	38.8628
0.2035	38.2711	38.4475	38.6990	38.9388	39.1364
0.2555	38.5284	38.6978	38.9500	39.1978	39.3997
0.3081	38.7259	38.9298	39.1712	39.3969	39.5612
0.3610	38.9355	39.1779	39.4161	39.6311	39.7689
0.4144	39.1305	39.4308	39.6659	39.8545	39.9620

(h) ***L- Serine- Urea- Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.1001	37.5835	37.9320	38.2809	38.5544	38.7481
0.2002	38.0870	38.3926	38.6681	38.8978	39.0736
0.3004	38.5450	38.4390	39.0462	39.2430	39.4161
0.4005	38.9605	39.2280	39.4188	39.5899	39.7603
0.5006	39.3594	39.6169	39.7896	39.9503	40.1101
0.6007	39.7452	39.9966	40.1548	40.3047	40.4576
0.7009	40.0750	40.3393	40.5101	40.6609	40.8187
0.8010	40.4142	40.6879	40.8714	40.0308	41.1855
0.9014	40.6962	40.9949	41.2226	41.4066	41.5543
1.0012	40.9557	41.2874	41.5678	41.7845	41.9329

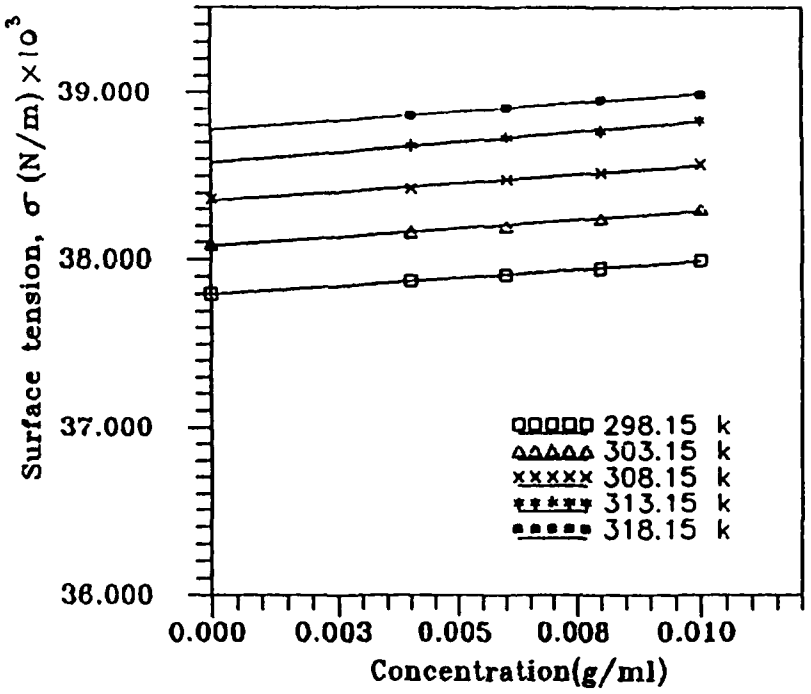

Fig 3.2(a) Plots of surface tension versus concentration for ovalbumin at pH 2.4

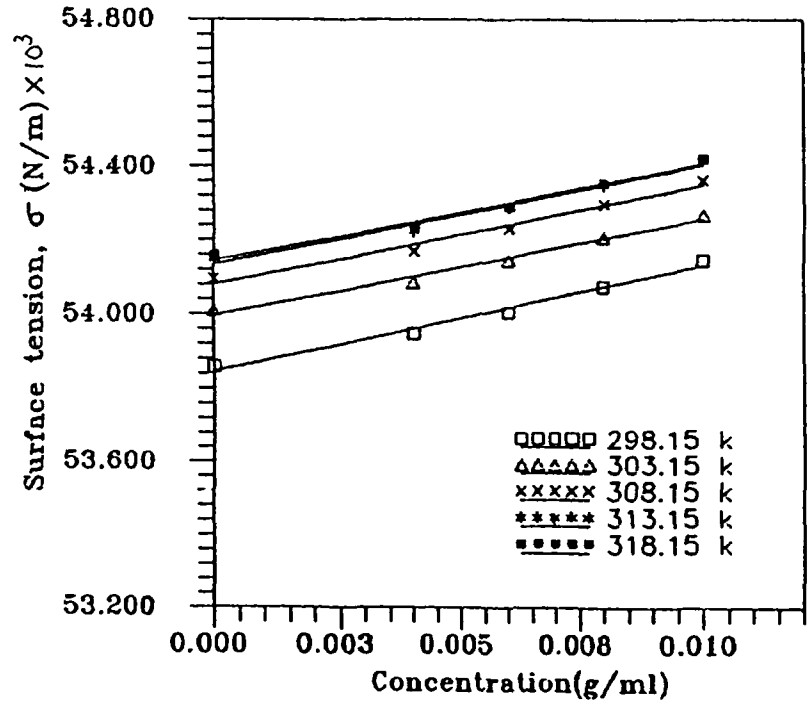

Fig 3.2(b) Plots of surface tension versus concentration for ovalbumin–maltose system at pH 2.4

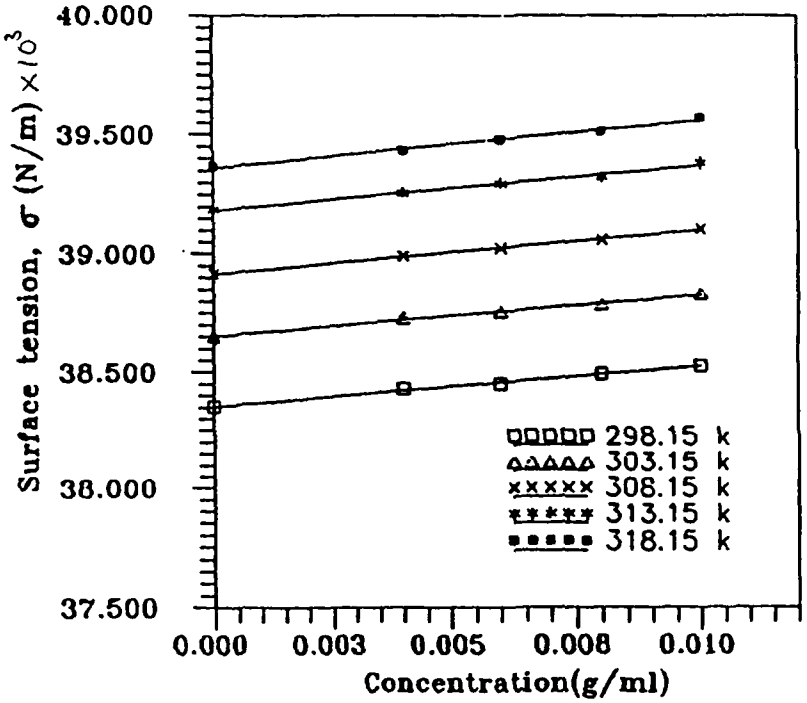

Fig 3.2(c) Plots of surface tension versus concentration for ovalbumin at pH 7.0

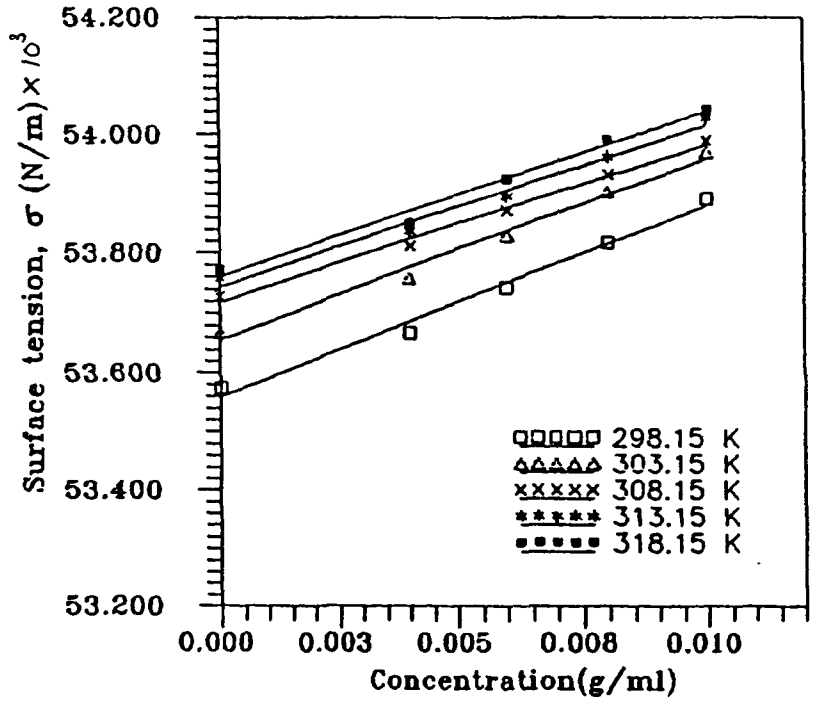

Fig 3.2(d) Plots of surface tension versus concentration for ovalbumin–maltose system at pH 7.0

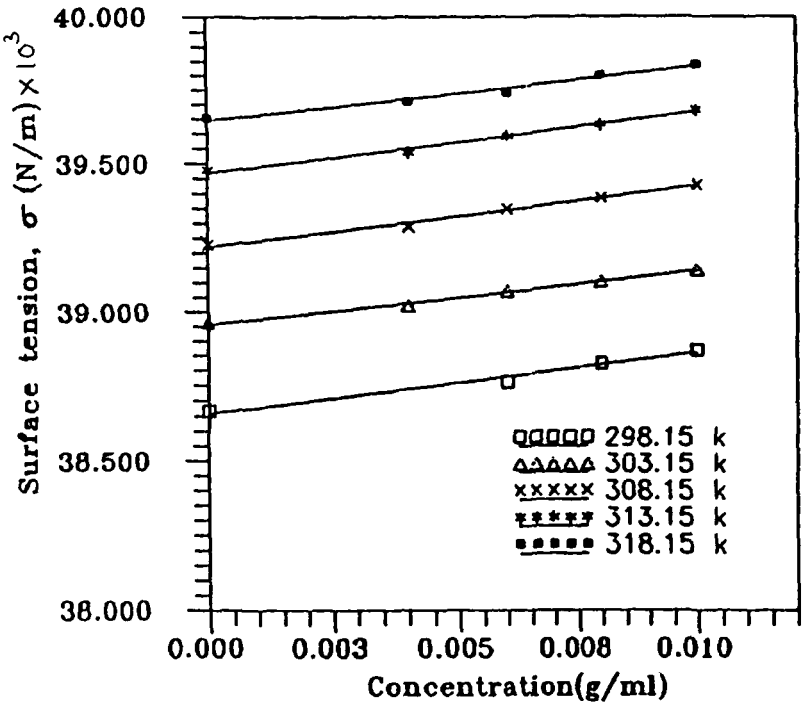

Fig 3.2(e) Plots of surface tension versus concentration for ovalbumin at pH 8.9

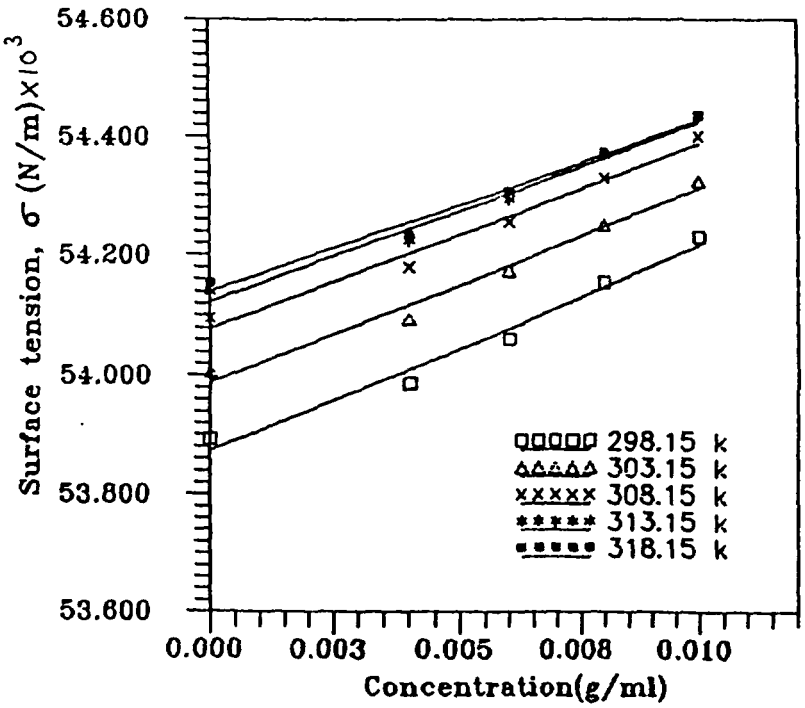

Fig 3.2(f) Plots of surface tension versus concentration for ovalbumin–maltose system at pH 8.9

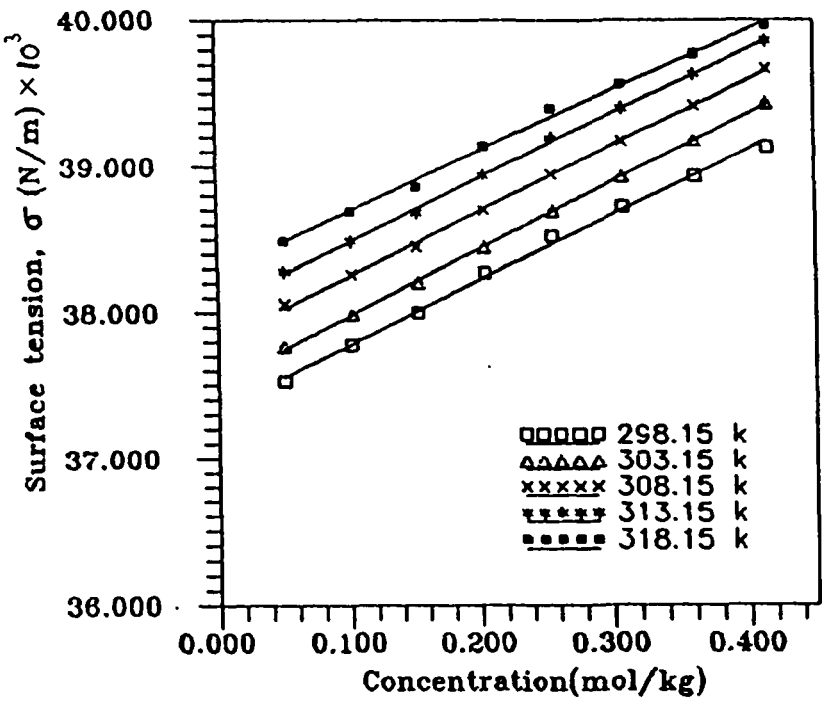

Fig 3.2(g) Plots of surface tension versus concentration for L-valine-urea-water system

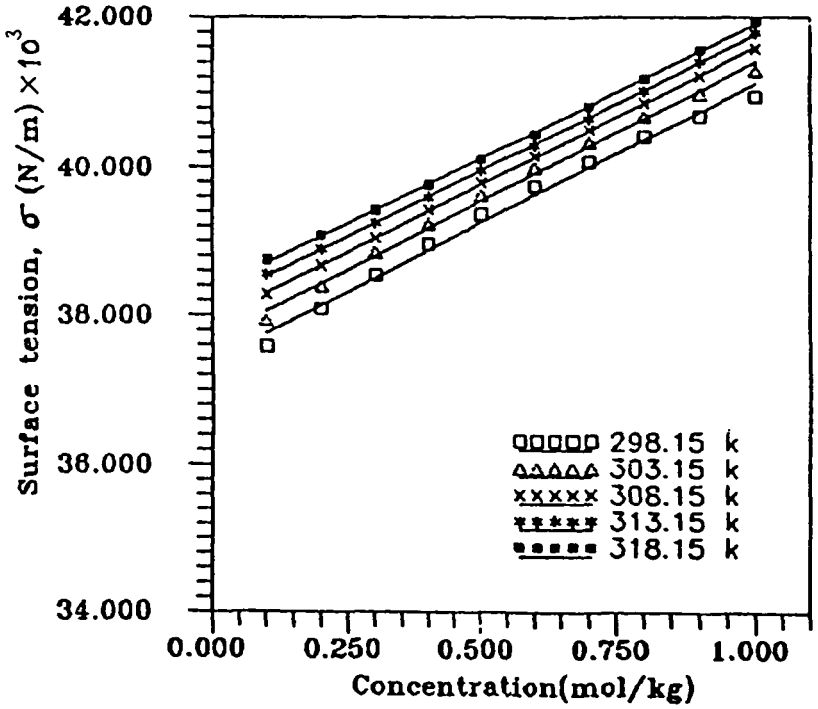

Fig 3.2(h) Plots of surface tension versus concentration for L-serine-urea-water system

CHAPTER -4

i) EFFECT OF MALTOSE ON THE STABILITY OF OVALBUMIN AS DEPICTED BY THE INTRINSIC VISCOSITY VALUES

ii) VISCOSITIES OF AMINO ACID-UREA-WATER MIXTURES

INTRODUCTION

The study of viscous behaviour of macromolecules in solutions is important in understanding the mechanism of transport processes. Viscosity and its derived parameters provide valuable information regarding the shapes and sizes of these molecules, as compact, globular or rod like particles or flexible random coils. The sensitivity of viscosity to molecular structure makes it useful for monitoring the processes that result in changes in the shapes and sizes of the molecules such as the denaturation of proteins, intermolecular cross-linking, intercalation of small molecules within the macromolecules etc.

The evaluation of intrinsic viscosity [168] has been used for the detection of the conformational changes in proteins. For native globular proteins, intrinsic viscosity, $[\eta]$, is of the order of 3-4 ml/g. It is independent of the molecular weight of protein. Its value goes on increasing as the protein undergoes denaturation. The shape factor, also determined from the viscosity measurement , is an important parameter providing information about the shape of the molecule during conformational changes.

The viscosity data may also be analyzed in terms of Jones-Dole equation. The viscosity B-coefficients provide a structure-breaking or structure-making effects of solutes on solvents. Therefore, in the present chapter, the viscosities of all the systems (described earlier) are reported as functions of temperature and concentration of the solutions. From these data, reduced viscosity, η_{red} , specific viscosity, η_{sp}, intrinsic viscosity, $[\eta]$ and the shape factor, ν, were evaluated. Viscosity B-coefficients were evaluated for amino acid-urea-water systems instead of shape factor and intrinsic viscosity.

THEORY

The temperature dependence of viscosity is given by the following polynomial equation

$$\eta = \Sigma^{2}_{i-0} \eta_i t^i \qquad 4.1$$

where t is in Kelvin. The experimental data of viscosity of pure solvent, η, and that of the solution, η', may be expressed in terms of specific viscosity,

$$\eta_{sp} = (\eta' - \eta)/\eta \qquad 4.2$$

The quantity η_{sp}, in the limit of infinite dilution, is proportional to the concentration, c, measured in grams per milliliter. Thus, the quantity η_{sp}/c called the reduced viscosity must be independent of concentration at zero concentration. This limiting value of η_{sp}/c is called the intrinsic viscosity, $[\eta]$,

$$[\eta] = \lim c \to 0\ \eta_{sp}/c = \lim c \to 0\ (\eta' - \eta)/\eta_C \qquad 4.3$$

$[\eta]$ is determined by measuring $(\eta'-\eta)/\eta_C$ at various concentrations and extrapolating to zero concentration. An equivalent result is obtained by measuring $1/c \ln \eta'/\eta$ and extrapolating to $c = 0$.

Since $\ln \eta'/\eta = \ln [1+ (\eta' -\eta)/\eta]$

in the limit of zero concentration $(\eta' - \eta)/\eta$ becomes very small so that the logarithm may be replaced by $(\eta' - \eta)/\eta$ i.e.,

$$\lim c \to 0\ 1/c \ln\ \eta'/\eta = \lim c \to 0\ (\eta' - \eta)/\eta_C \qquad 4.4$$

The intrinsic viscosity of a protein can also be expressed in terms of partial specific volume [168]

$$[\eta] = \nu(\bar{v}^0 + \Sigma\, m_i\, v_i^0) \qquad 4.5$$

where ν is the Simha's or shape factor, m_i is the mass of solvent in grams with partial specific volume v_i^0 bound to lg dry weight of protein. The value of ν is 2.5 for spheres and larger for ellipsoids. If the native ovalbumin in phosphate buffer binds only with water for which v_i^0 may be taken to be 1, the maximum amount of water bound to 1g of protein (spherical) may be calculated by setting $\nu = 2.5$ in

equation 4.5. Thus, the value of $\Sigma\, m_i\, v_i^0$ turns out to be 0.29. Using this value in equation 4.5, the shape factor can be calculated.

The viscosity B-coefficient of the Jones-Dole equation [118] has been determined according to the equation

$$\eta_r = \eta'/\eta = 1 + Ac^{1/2} + Bc + Dc^2 \qquad 4.6$$

where c is the molar concentration (moles/liter); A, B and D are the coefficients to be determined. A- coefficients possess a non-zero value only for electrolytes where they are observed to be always positive, B-coefficients are positive for non-electrolytes and either positive or negative for electrolytes. B-coefficients presumably measure the size and shape effect of the solute as well as the structural modification induced by solute- solvent interaction [101]. The significance of D-coefficients is not clearly understood.

RESULTS AND DISCUSSION

The coefficients of equation 4.1 are given in tables 4.1 along with the standard deviation. The experimental values of viscosity of the systems under investigation are plotted as functions of concentration and temperature in figs. 4.1 (a-h). The plots show an increase in the values of viscosity with an increase in the concentration of the solution while an inverse relation is observed with temperature. The increase in the concentration of the solute increases the frictional forces (the attractive forces) between the neighbouring portions of the solution and therefore, increases its viscosity. When maltose is added to the protein solution there is 6 to 8 times increase in the viscosity of solutions (tables 4.2 a-f).

The values of specific viscosity, η_{sp} and the reduced viscosity, η_{red}, are plotted as functions of concentration and temperature (figs 4.2 and 4.3). The specific viscosity is the concentration dependent quantity while the reduced viscosity does not show any concentration dependence. η_{sp} increases with the

Table 4.1: Least-squares fit parameters of equation 4.1 for the following systems:

(a) Ovalbumin- Buffer System (pH 2.4)

Concentration g/ml	η_0	η_1	$\eta_2 x 10^3$	Standard Devn.($x10^3$)
0.000	293.849	−1.71327	2.54000	09.6878
0.004	290.075	−1.68269	2.48314	04.1494
0.006	293.408	−1.70160	2.51057	09.1393
0.008	302.340	−1.75883	2.60285	12.0150
0.010	297.406	−1.72668	2.55114	11.1103

(b) Ovalbumin-Maltose-Buffer System (pH 2.4)

Concentration g/ml	η_0	η_1	$\eta_2 x 10^3$	Standard Devn.($x10^3$)
0.000	3649.09	−21.4911	3.18940	2.8681
0.004	3684.61	−21.7051	3.22234	4.9812
0.006	3751.56	−22.1230	3.28800	4.9434
0.008	3772.06	−22.2491	3.30794	0.5399
0.010	3940.43	−23.3344	3.48334	3.3220

(c) Ovalbumin- Buffer System (pH 7.0)

Concentration g/ml	η_0	η_1	$\eta_2 x 10^3$	Standard Devn.($x10^3$)
0.000	311.353	−1.80965	2.67371	06.6554
0.004	315.870	−1.83724	2.71686	08.9856
0.006	299.273	−1.72831	2.53886	01.0007
0.008	297.372	−1.71550	2.51800	02.8135
0.010	321.855	−1.87209	2.76885	11.3520

(d) ***Ovalbumin-Maltose-Buffer System*** *(pH 7.0)*

Concentration g/ml	η_0	η_1	$\eta_2 x10^3$	Standard Devn.($x10^3$)
0.000	4173.31	-24.9091	3.74260	09.6192
0.004	4226.87	-25.2280	3.79291	11.9458
0.006	4348.96	-26.0045	3.91712	09.7183
0.008	4354.99	-26.0186	3.91606	05.8034
0.010	4268.85	-25.4618	3.82683	10.6029

(e) ***Ovalbumin-Buffer System*** *(pH 8.9)*

Concentration g/ml	η_0	η_1	$\eta_2 x10^3$	Standard Devn.($x10^3$)
0.000	338.456	-1.98093	2.94543	04.7802
0.004	315.323	-1.82771	2.69371	12.7323
0.006	308.142	-1.78112	2.61886	17.1100
0.008	332.934	-1.94091	2.87686	12.4775
0.010	308.689	-1.78215	2.61772	11.6611

(f) ***Ovalbumin-Maltose-Buffer System*** *(pH 8.9)*

Concentration g/ml	η_0	η_1	$\eta_2 x10^3$	Standard Devn.($x10^3$)
0.000	4836.84	-29.1698	4.43003	0.1285
0.004	4776.89	-28.7624	4.36197	0.1308
0.006	4617.18	-27.7254	4.19440	0.1265
0.008	4407.59	-26.3576	3.97197	0.0959
0.010	4439.13	-26.5495	4.00177	0.1480

(g) ***L-Valine-Urea-Water System***

Concentration mol/kg	η_0	η_1	$\eta_2 x10^3$	Standard Devn.($x10^3$)
0.0000	316.898	−1.85868	2.77057	02.5567
0.0502	314.196	−1.83959	2.73828	07.1826
0.1008	311.711	−1.81944	2.70029	09.5927
0.1502	333.264	−1.95113	2.90257	06.8537
0.2035	332.391	−1.94199	2.88371	03.5039
0.2555	330.618	−1.92520	2.84971	05.1919
0.3081	348.290	−2.03218	3.01257	07.1737
0.3610	361.305	−2.10966	3.12886	11.0495
0.4144	377.799	−2.20907	3.27971	15.4170

(h) ***L-Serine-Urea-Water System***

Concentration mol/kg	η_0	η_1	$\eta_2 x10^3$	Standard Devn.($x10^3$)
0.1001	257.031	−1.47073	2.14343	06.9666
0.2002	262.459	−1.49829	2.17800	02.2289
0.3004	288.513	−1.66193	2.43657	07.3571
0.4005	300.461	−1.73439	2.54771	09.8785
0.5006	322.468	−1.87303	2.76771	10.5314
0.6007	321.825	−1.86137	2.73886	09.4246
0.7009	341.121	−1.97849	2.91772	09.0472
0.8010	309.938	−1.77428	2.58571	07.1983
0.9014	342.909	−1.98076	2.91057	08.3170
1.0012	356.738	−2.06442	3.03857	08.6136

Table 4.2: Experimental values of viscosity ($\eta x10^4$ $kgm^{-1}s^{-1}$) as functions of temperature and concentration for the following systems:

(a) Ovalbumin- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	8.8329	7.8865	7.0968	6.4278	5.8651
0.004	9.1148	8.1736	7.3402	6.6467	6.0701
0.006	9.2499	8.2912	7.4448	6.7573	6.1585
0.008	9.3256	8.3507	7.5052	6.8233	6.2221
0.010	9.3791	8.4148	7.5671	6.8845	6.2841

(b) Ovalbumin-Maltose-Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	76.7010	65.1001	55.1912	46.7590	40.0012
0.004	77.6901	65.9695	56.0498	47.5455	40.7563
0.006	78.4100	66.6510	56.5895	48.0012	41.2596
0.008	79.0486	67.2540	57.1125	48.6404	41.8000
0.010	79.7509	67.8015	57.6412	49.1016	42.4377

(c) Ovalbumin-Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	9.4844	8.4619	7.5979	6.8569	6.2408
0.004	9.6125	8.5773	7.7115	6.9670	6.3466
0.006	9.6653	8.6555	7.7745	7.0215	6.3920
0.008	9.7260	8.7242	7.8367	7.0831	6.4550
0.010	9.8291	8.7737	7.8904	7.1420	6.5037

(d) ***Ovalbumin-Maltose-Buffer System (pH 7.0)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	75.7915	63.6500	53.6290	45.4979	38.9610
0.004	76.8654	64.5790	54.4705	46.3164	39.6904
0.006	77.8050	65.4909	55.1498	47.0224	40.4564
0.008	78.6666	66.2801	55.8470	47.5343	40.9407
0.010	79.2207	67.0010	56.5000	48.3187	41.6361

(e) ***Ovalbumin-Buffer System (pH 8.9)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	09.6683	8.6275	7.7144	6.9652	6.3579
0.004	09.8369	8.8216	7.8892	7.1238	6.4964
0.006	09.8925	8.8924	7.9524	7.1964	6.5626
0.008	09.9781	8.9470	8.0113	7.2439	6.6321
0.010	10.0339	9.0119	8.0906	7.2979	6.6697

(f) ***Ovalbumin-Maltose-Buffer System (pH 8.9)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.000	77.9485	65.0603	54.7932	46.6638	40.4593
0.004	78.9768	66.0671	55.7813	47.5506	41.2468
0.006	79.4577	66.6949	56.4900	48.2013	41.8206
0.008	79.9323	67.3781	57.2046	48.7490	42.2868
0.010	80.7904	68.0733	57.9665	49.4463	42.9390

(g) ***L-Valine-Urea-Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.0000	09.0159	8.0561	7.2250	6.5424	5.9932
0.0502	09.1333	8.1784	7.3447	6.6430	6.1013
0.1008	09.2857	8.2952	7.4549	6.7625	6.1706
0.1520	09.5504	8.5304	7.6428	6.8931	6.3121
0.2035	09.7278	8.6941	7.7930	7.0390	6.4364
0.2555	09.9379	8.8896	7.9639	7.1902	6.5646
0.3081	10.1890	9.0988	8.1341	7.3284	6.6859
0.3610	10.4378	9.3166	8.3149	7.4795	6.8227
0.4144	10.7012	9.5440	8.5010	7.6329	6.9622

(h) ***L-Serine-Urea-Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.1001	09.0657	08.1673	7.3582	6.6547	6.0786
0.2002	09.3462	08.4243	7.5902	6.8214	6.2480
0.3004	09.6006	08.6261	7.7602	7.0079	6.4030
0.4005	09.8239	08.8223	7.9359	7.1602	6.5491
0.5006	10.0493	09.0207	8.1070	7.3250	6.7149
0.6007	10.3201	09.2599	8.3197	7.5059	6.8618
0.7009	10.5955	09.4878	8.5075	7.6654	6.9992
0.8010	10.7863	09.7004	8.7249	7.8776	7.1801
0.9014	11.0711	09.9306	8.9157	8.0427	7.3406
1.0012	11.3351	10.1609	9.1199	8.2248	7.5094

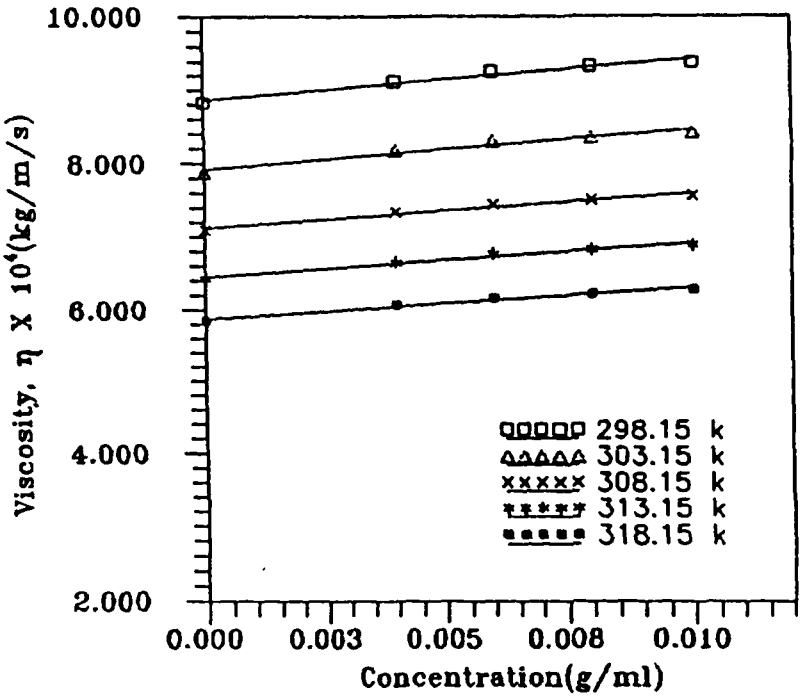

Fig 4.1(a) Plots of viscosity versus concentration for ovalbumin at pH 2.4

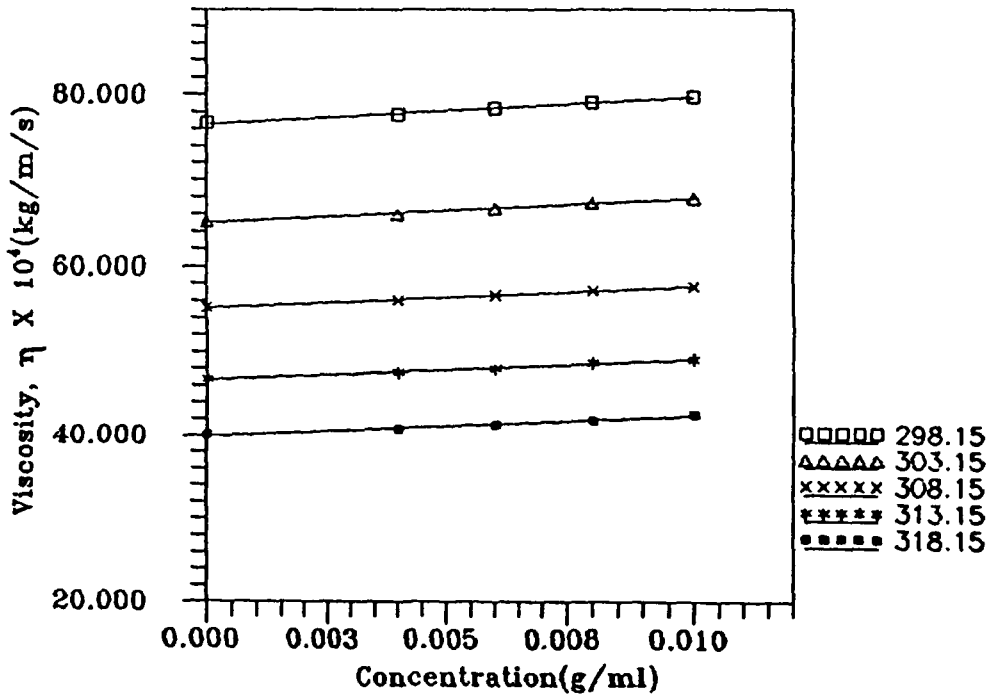

Fig 4.1(b) Plots of viscosity versus concentration for ovalbumin–maltose system at pH 2.4

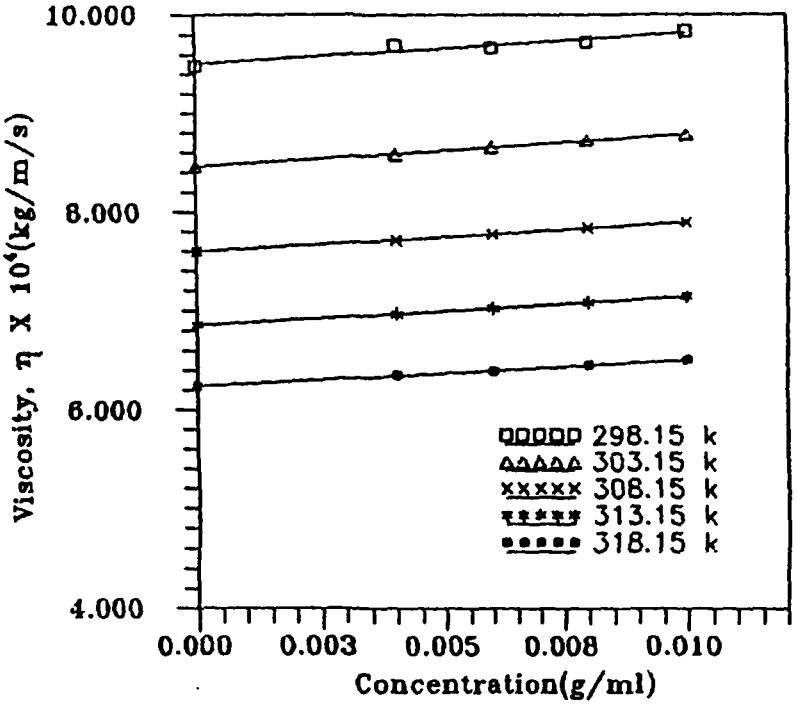

Fig 4.1(c) Plots of viscosity versus concentration for ovalbumin at pH 7.0

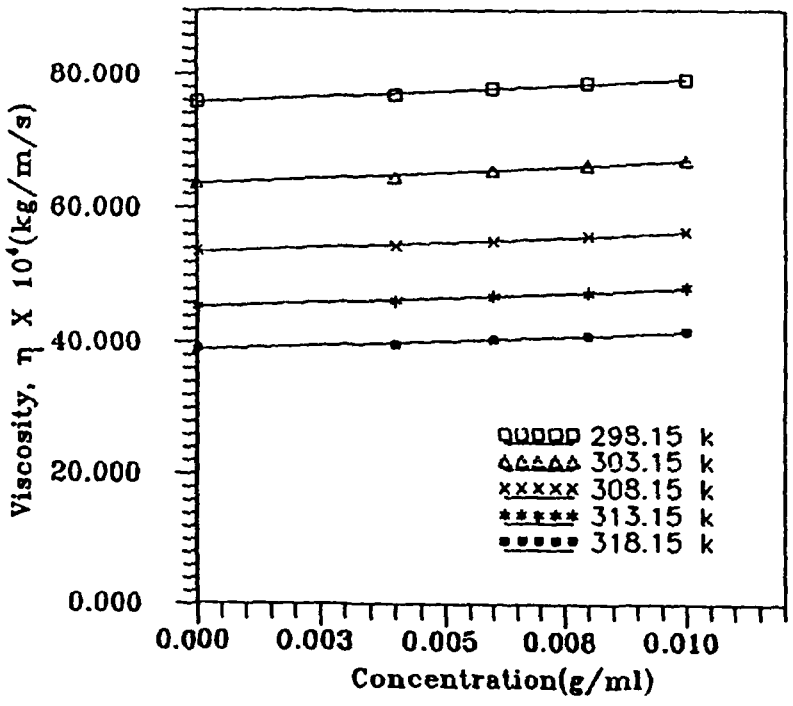

Fig 4.1(d) Plots of viscosity versus concentration for ovalbumin–maltose system at pH 7.0

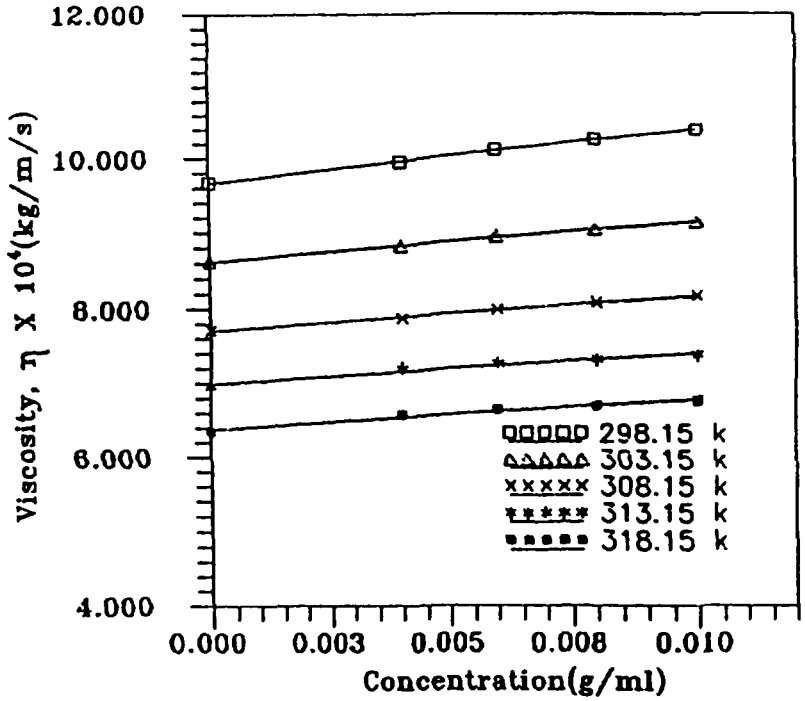

Fig 4.1(e) Plots of viscosity versus concentration for ovalbumin at pH 8.9

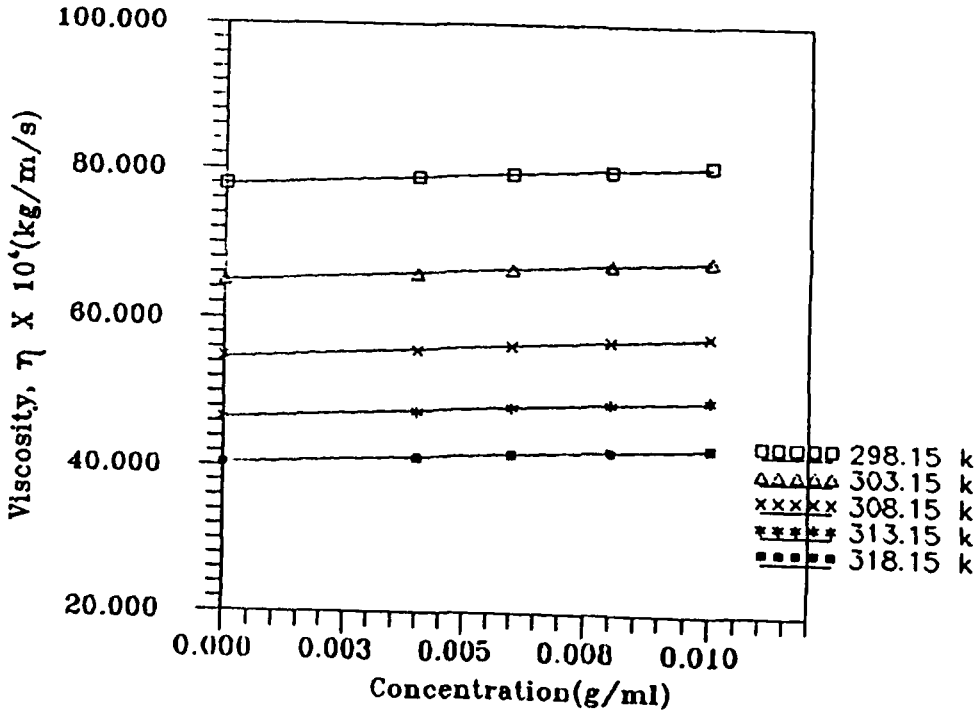

Fig 4.1(f) Plots of viscosity versus concentration for ovalbumin–maltose system at pH 8.9

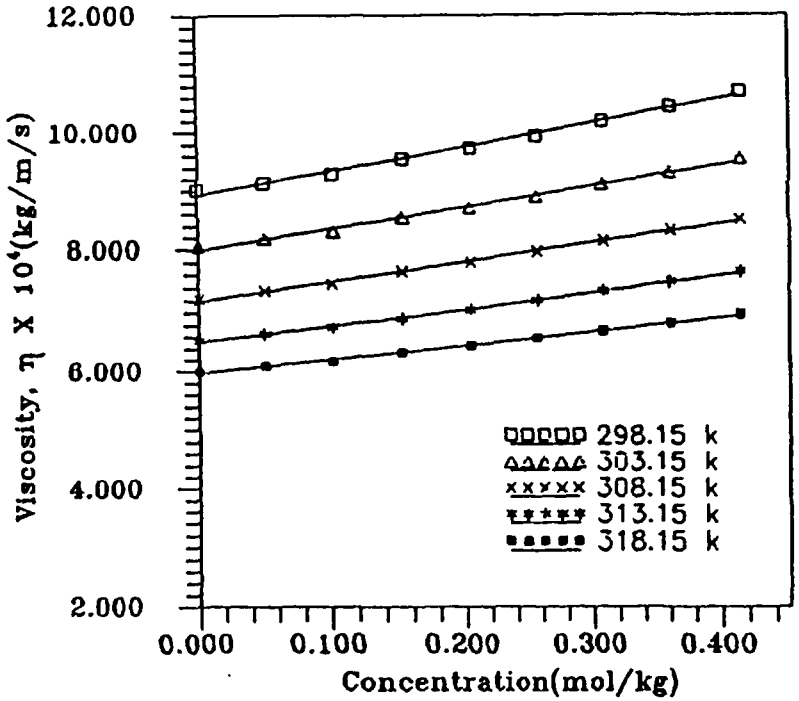

Fig 4.1(g) Plots of viscosity versus concentration for L-valine-urea-water system

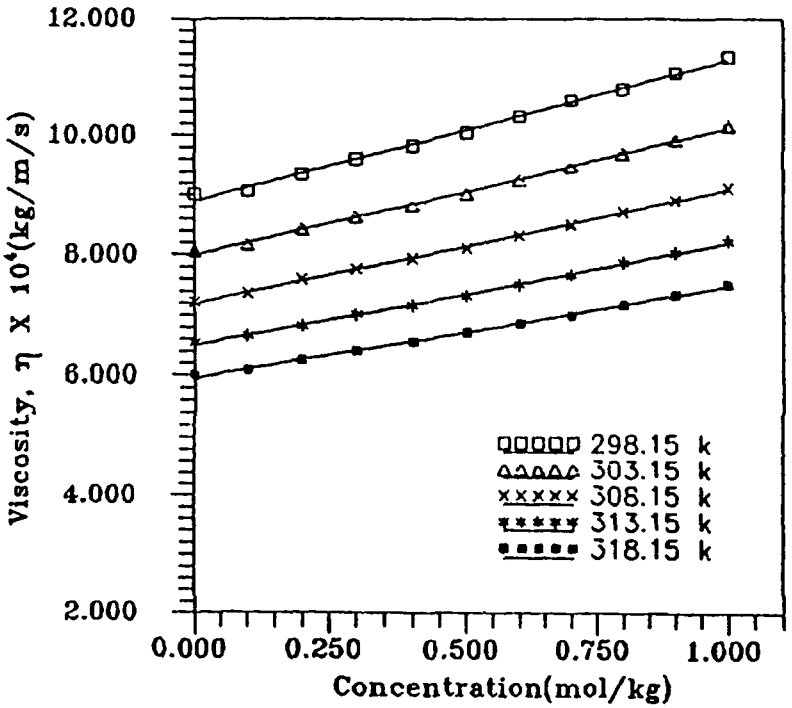

Fig 4.1(h) Plots of viscosity versus concentration for L-serine-urea-water system

Table 4.3: Specific Viscosity, η_{sp}, as functions of temperature and concentration for the following systems:

(a) Ovalbumin- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.0319	0.0365	0.0343	0.0341	0.0367
0.006	0.0472	0.0514	0.0490	0.0513	0.0518
0.008	0.0558	0.0590	0.0575	0.0615	0.0627
0.010	0.0618	0.0671	0.0663	0.0711	0.0733

(b) Ovalbumin-Maltose-Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.0129	0.0134	0.0156	0.0168	0.0189
0.006	0.0223	0.0238	0.0253	0.0266	0.0315
0.008	0.0306	0.0331	0.0348	0.0402	0.0450
0.010	0.0398	0.0415	0.0444	0.0501	0.0609

(c) Ovalbumin-Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.0135	0.0136	0.0150	0.0161	0.0170
0.006	0.0191	0.0229	0.0232	0.0240	0.0242
0.008	0.0255	0.0310	0.0314	0.0330	0.0343
0.010	0.0363	0.0368	0.0385	0.0416	0.0421

(d) Ovalbumin-Maltose-Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.0142	0.0146	0.0157	0.0180	0.0187
0.006	0.0266	0.0289	0.0284	0.0335	0.0384
0.008	0.0379	0.0413	0.0414	0.0448	0.0508
0.010	0.0452	0.0526	0.0535	0.0620	0.0687

(e) Ovalbumin-Buffer System (pH 8.9)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.0174	0.0225	0.0227	0.0228	0.0218
0.006	0.0232	0.0307	0.0309	0.0332	0.0322
0.008	0.0320	0.0370	0.0385	0.0400	0.0431
0.010	0.0378	0.0446	0.0488	0.0478	0.0490

(f) Ovalbumin-Maltose-Buffer System (pH 8.9)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	0.0132	0.0155	0.0180	0.0190	0.0195
0.006	0.0194	0.0251	0.0310	0.0329	0.0336
0.008	0.0255	0.0356	0.0440	0.0447	0.0452
0.010	0.0365	0.0463	0.0579	0.0596	0.0613

(g) ***L-Valine-Urea-Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.0502	0.0130	0.0152	0.0166	0.0154	0.0180
0.1008	0.0299	0.0297	0.0318	0.0336	0.0296
0.1520	0.0593	0.0589	0.0578	0.0536	0.0532
0.2035	0.0790	0.0792	0.0786	0.0790	0.0740
0.2555	0.1023	0.1035	0.1023	0.0990	0.0953
0.3081	0.1301	0.1294	0.1258	0.1201	0.1155
0.3610	0.1577	0.1565	0.1509	0.1432	0.1384
0.4144	0.1869	0.1847	0.1766	0.1666	0.1616

(h) ***L-Serine-Urea-Water System***

Concentration mol/kg	Temperature K 298.15	303.15	308.15	313.15	318.15
0.1001	0.0052	0.0138	0.0184	0.0172	0.0142
0.2002	0.0366	0.0457	0.0505	0.0426	0.0425
0.3004	0.0649	0.0708	0.0741	0.0712	0.0684
0.4005	0.0896	0.0951	0.0984	0.0944	0.0928
0.5006	0.1146	0.1197	0.1221	0.1196	0.1204
0.6007	0.1447	0.1494	0.1515	0.1473	0.1449
0.7009	0.1752	0.1777	0.1775	0.1716	0.1679
0.8010	0.1964	0.2041	0.2076	0.2040	0.1980
0.9014	0.2280	0.2326	0.2340	0.2293	0.2248
1.0012	0.2572	0.2613	0.2623	0.2572	0.2530

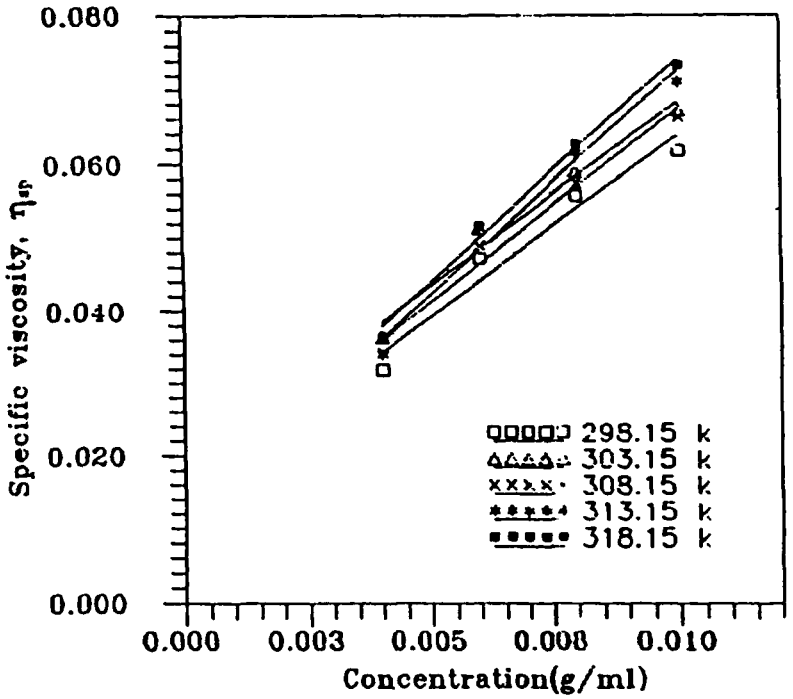

Fig 4.2(a) Plots of specific viscosity versus concentration for ovalbumin at pH 2.4

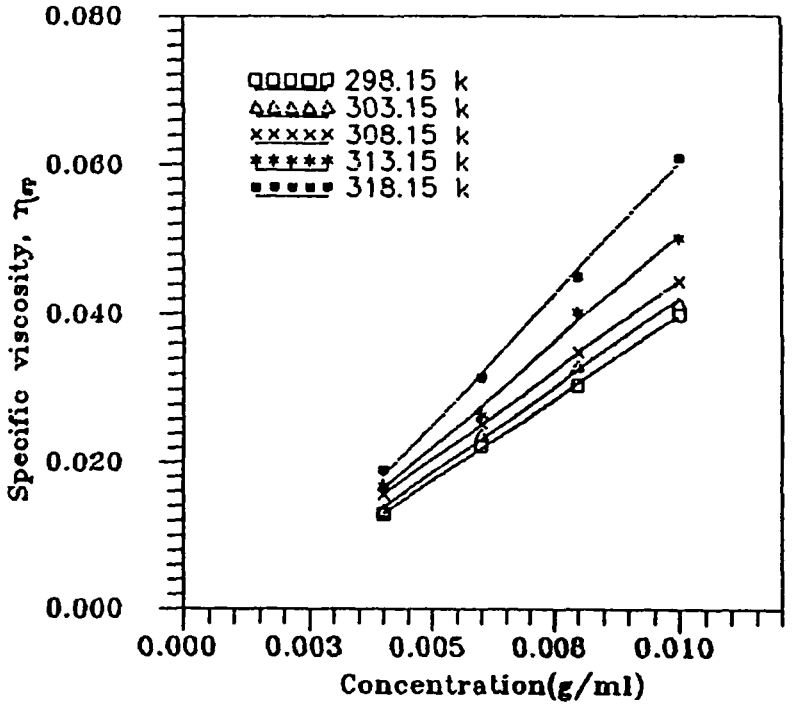

Fig 4.2(b) Plots of specific viscosity versus concentration for ovalbumin–maltose system at pH 2.4

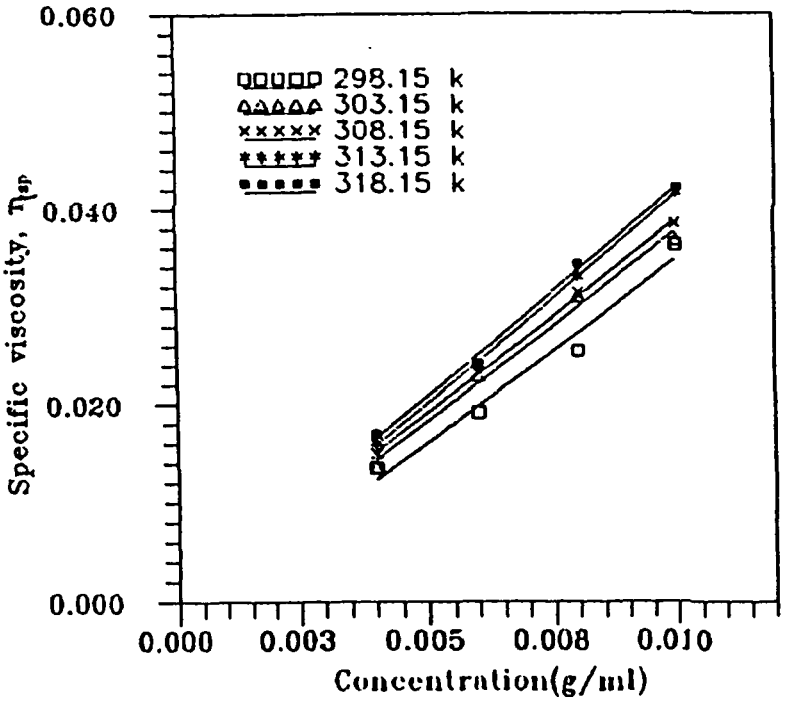

Fig 4.2(c) Plots of specific viscosity versus concentration for ovalbumin at pH 7.0

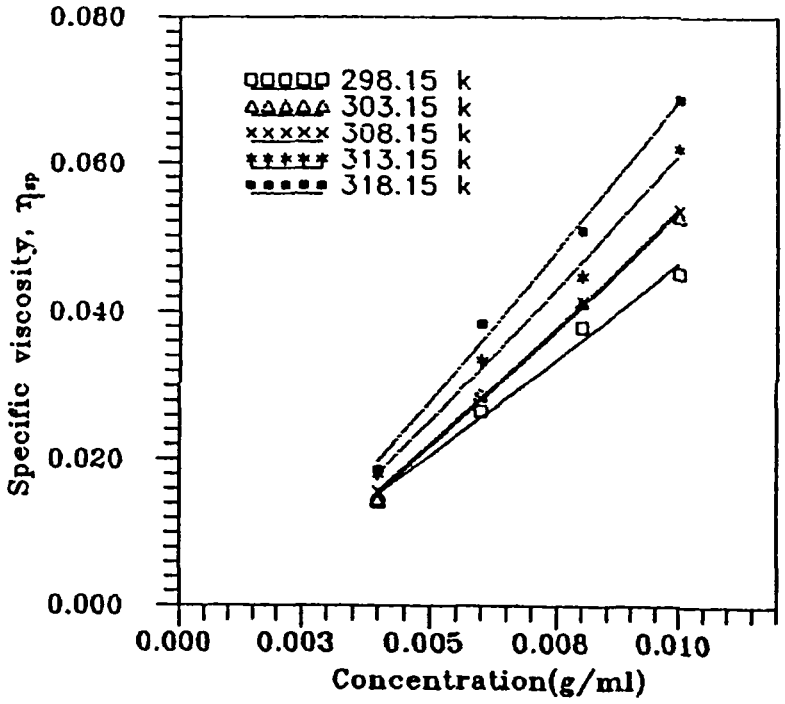

Fig 4.2(d) Plots of specific viscosity versus concentration for ovalbumin–maltose system at pH 7.0

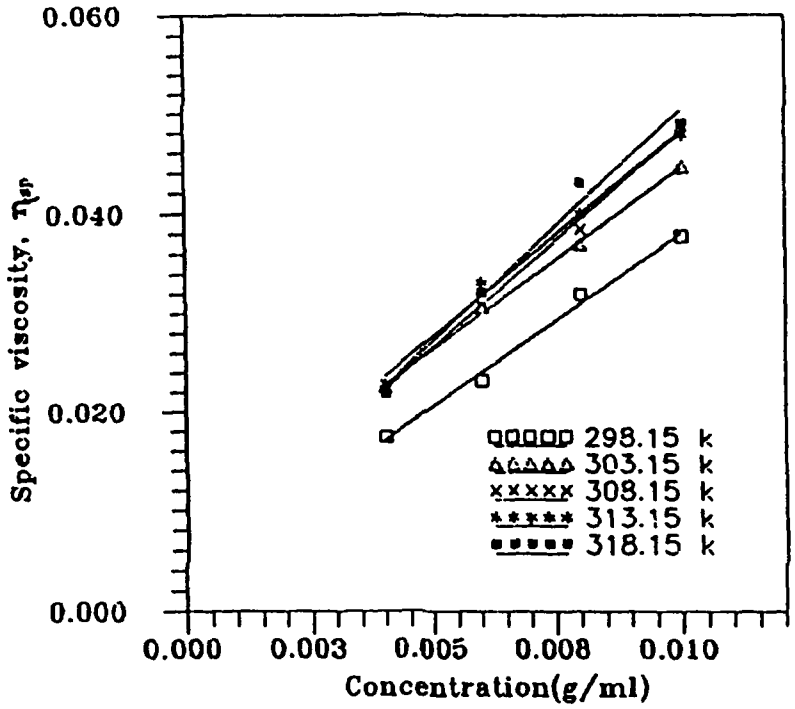

Fig 4.2(e) Plots of specific viscosity versus concentration for ovalbumin at pH 8.9

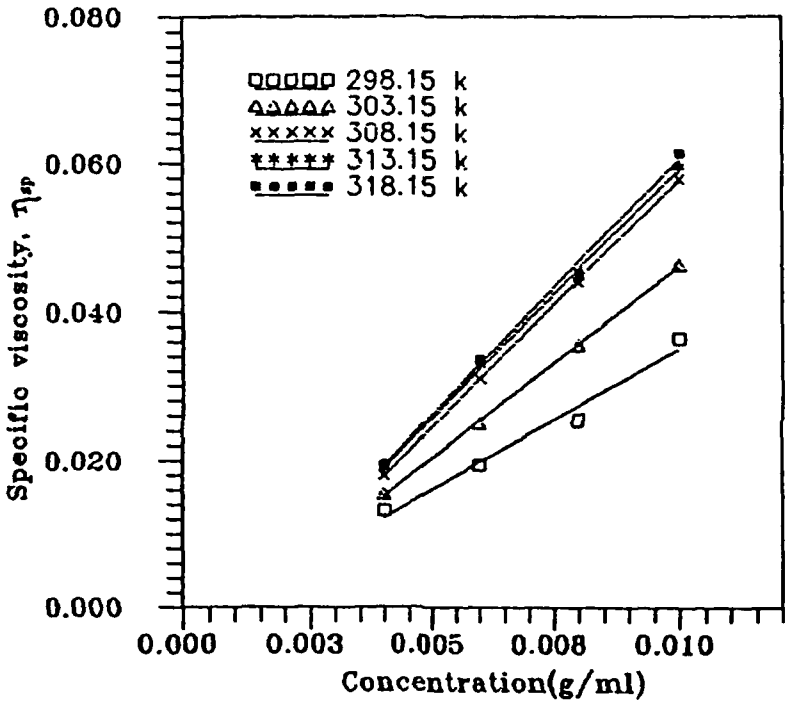

Fig 4.2(f) Plots of specific viscosity versus concentration for ovalbumin–maltose system at pH 8.9

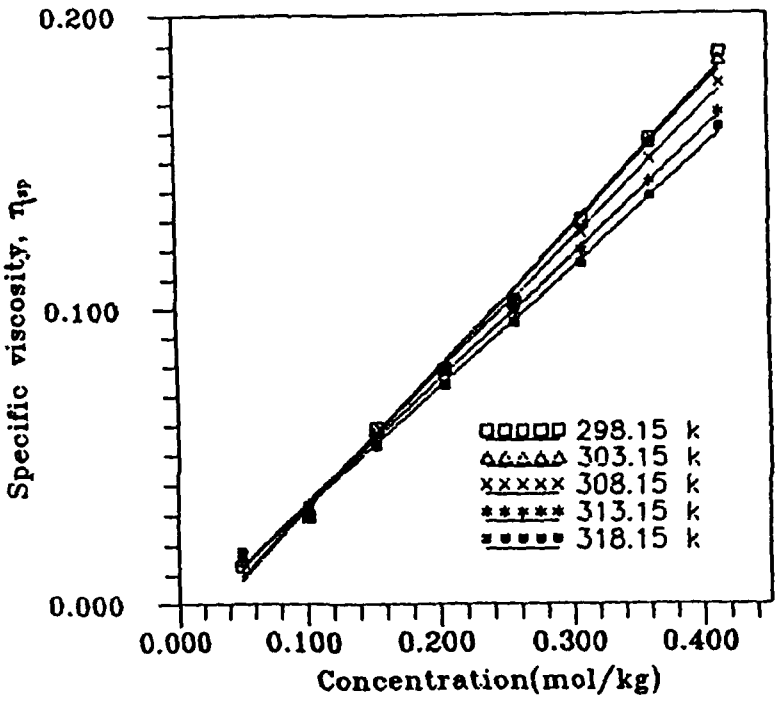

Fig 4.2(g) Plots of specific viscosity versus concentration for L-valine-urea-water system

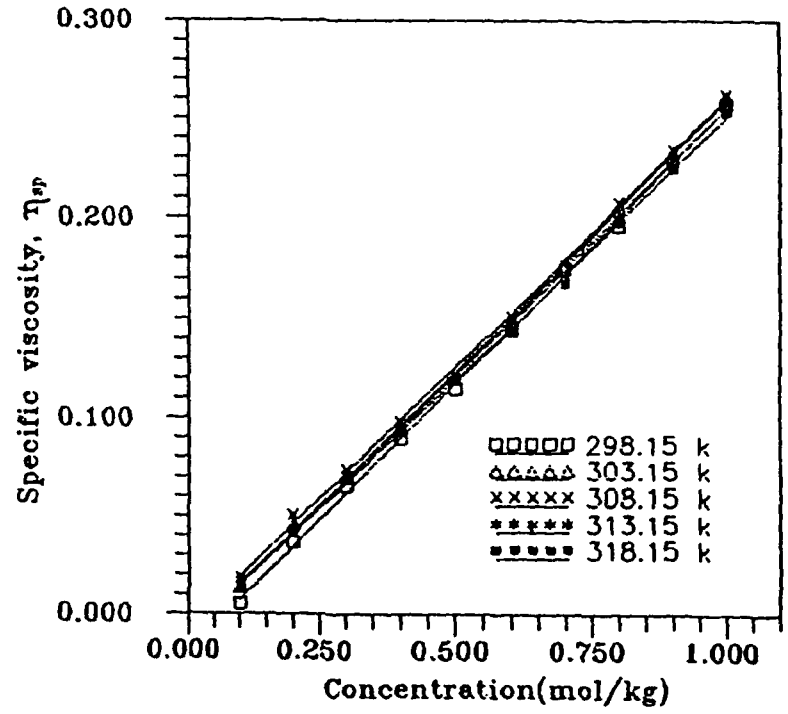

Fig 4.2(h) Plots of specific viscosity versus concentration for L-serine-urea-water system

Table 4.4: Reduced Viscosity, (η_{red}, ml/g) as functions of temperature and concentration for the following systems:

(a) Ovalbumin- Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	7.8044	8.7182	8.3110	8.4106	8.8023
0.006	7.7508	8.2423	7.8000	8.3414	8.3212
0.008	6.8850	7.0832	7.0176	7.3606	7.4525
0.010	6.0425	6.2636	6.4098	6.7644	6.9220

(b) Ovalbumin-Maltose-Buffer System (pH 2.4)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	3.2033	3.5100	3.8590	4.3701	4.8752
0.006	3.6728	3.9240	4.1700	4.3699	5.1623
0.008	3.7685	4.0688	4.2774	4.9310	5.4984
0.010	3.6608	4.0658	4.3434	4.8885	5.8126

(c) Ovalbumin-Maltose-Buffer System (pH 7.0)

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	3.3540	3.3864	3.7102	3.9823	4.2018
0.006	3.1490	3.7702	3.8295	3.9536	4.0818
0.008	3.1443	3.8173	3.8682	3.9590	4.2065
0.010	3.5699	3.6185	3.7775	3.9780	4.2850

(d) ***Ovalbumin-Maltose-Buffer System (pH 7.0)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	3.5174	3.7225	3.9923	4.4575	4.8370
0.006	4.3699	5.1324	4.6605	5.4930	6.2773
0.008	4.6541	5.0615	5.0657	5.4732	6.1954
0.010	4.4251	5.1308	5.2051	6.0512	6.6406

(e) ***Ovalbumin-Buffer System (pH 8.9)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	5.6240	7.1311	7.6732	8.4874	8.5545
0.006	4.9248	5.5023	6.3702	6.5829	6.9724
0.008	4.5690	5.2416	6.0214	6.1416	6.5500
0.010	4.3507	4.9672	5.8024	6.0032	6.2856

(f) ***Ovalbumin-Maltose-Buffer System (pH 8.9)***

Concentration g/ml	Temperature K 298.15	303.15	308.15	313.15	318.15
0.004	3.2764	3.8391	4.4681	4.7064	4.8193
0.006	3.1961	4.1357	5.0829	5.4028	5.5154
0.008	3.1415	4.3757	5.3835	5.4645	5.5223
0.010	3.5810	4.5271	5.6299	5.7919	5.9484

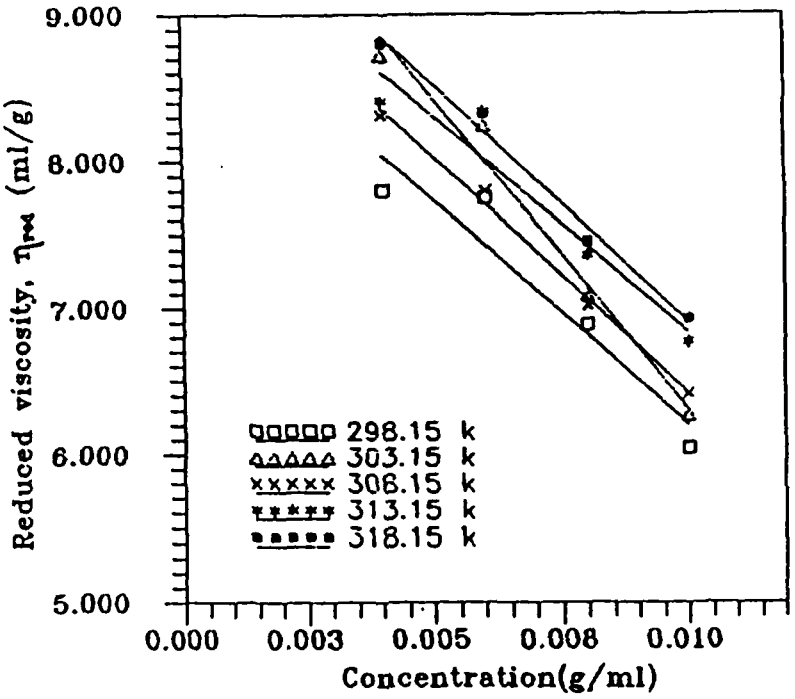

Fig 4.3(a) Plots of reduced viscosity versus concentration for ovalbumin at pH 2.4

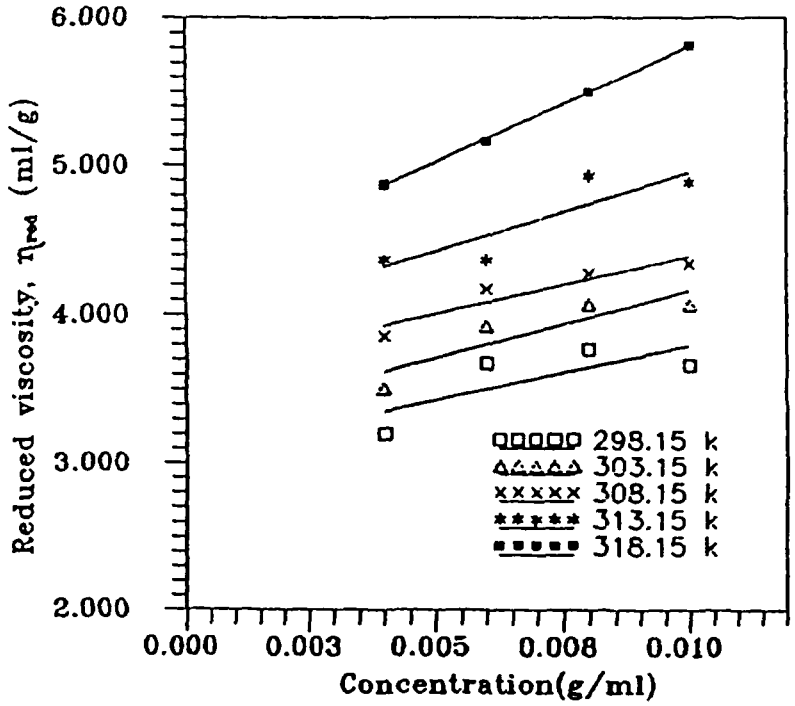

Fig 4.3(b) Plots of reduced viscosity versus concentration for ovalbumin–maltose system at pH 2.4

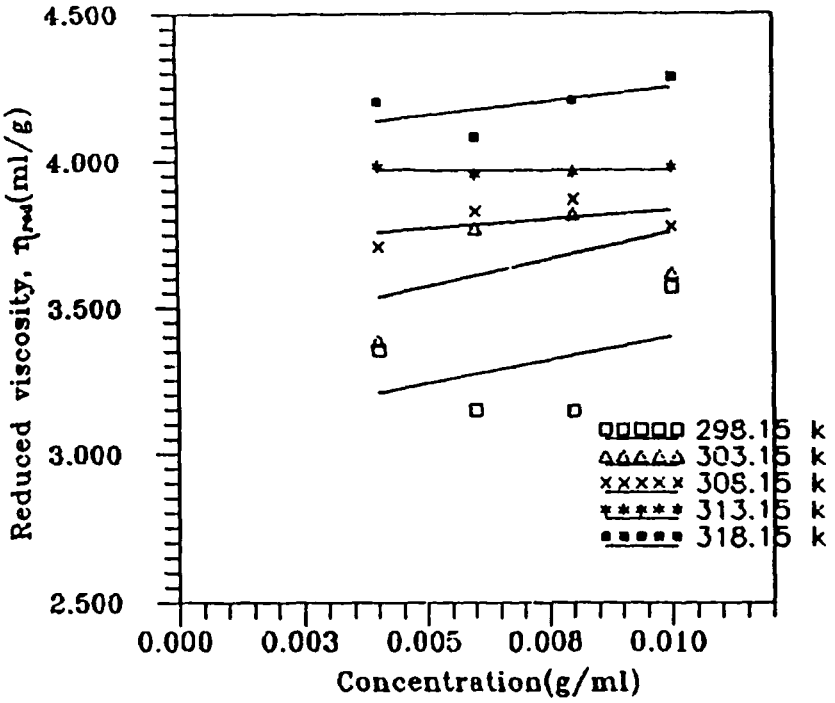

Fig 4.3(c) Plots of reduced viscosity versus concentration for ovalbumin at pH 7.0

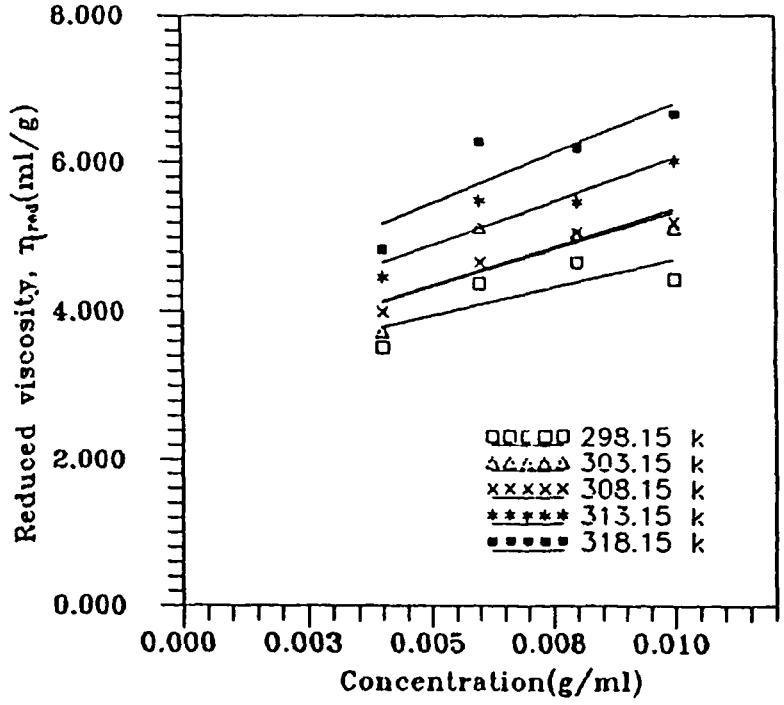

Fig 4.3(d) Plots of reduced viscosity versvs concentration for ovalbumin–maltose system at pH 7.0

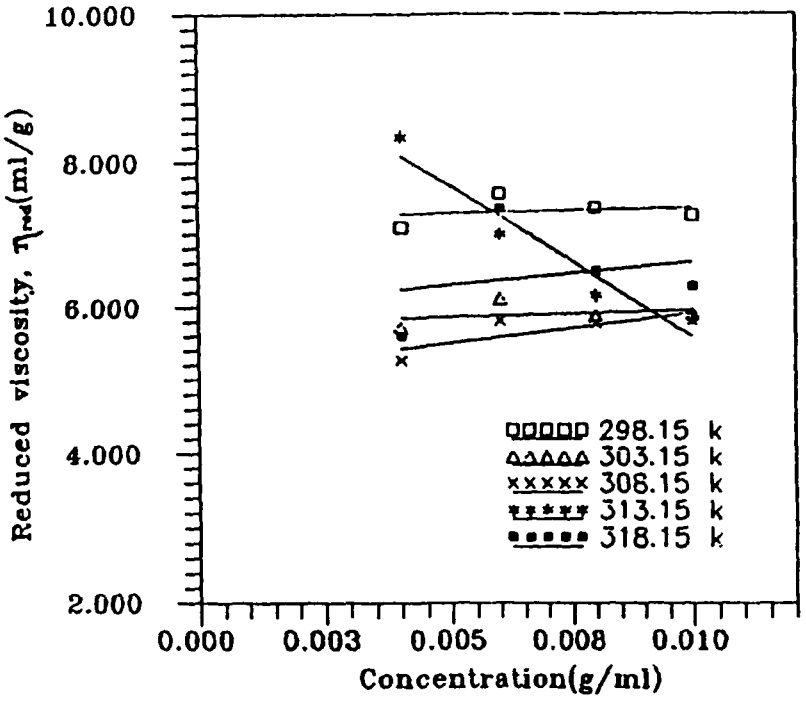

Fig 4.3(e) Plots of reduced viscosity versus concentration for ovalbumin at pH 8.9

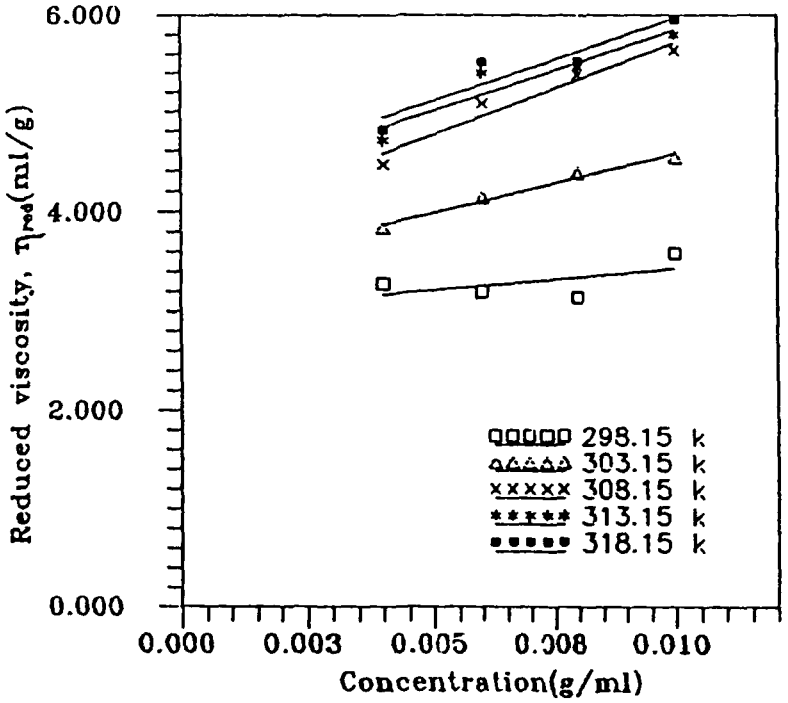

Fig 4.3(f) Plots of reduced viscosity versus concentration for ovalbumin–maltose system at pH 8.9

increase in concentration and temperature while η_{red} increases with the increase in temperature only.

The intrinsic viscosity is obtained by plotting the reduced viscosity as a function of concentration and extrapolating it to c=0, so the intrinsic viscosity is the reduced viscosity at infinite dilution. An examination of the tables 4.5 (a-b) show that the value of $[\eta]$ lies between 3-4 ml/g in the temperature range of 25.0° to 40.0° C at pH 7.0. This represents the native state of ovalbumin. The value of $[\eta]$ increases with the increase in temperature of the system and its value goes beyond 4 ml/g after 40.0° C. This shows that denaturation has just started.

Proteins are stabilized generally by a combination of hydrogen bonding, electrostatic interactions and hydrophobic interactions with additional contributions in particular proteins from cross-linking, metal complexing etc. Of all these, the hydrophobic interactions provide the major contribution to stabilizing the globular form of most soluble proteins. In discussing the effect of maltose on the stability of ovalbumin, we have to consider the effects of maltose sugar on these various forces and interactions.

In aqueous solutions of proteins there is a cooperative hydrogen bonded structure [8] in which water competes as both donor and acceptor with backbone and side chain groups in the protein. When sugar is added to the protein solution the individual OH groups of sugar may also compete for hydrogen bonding but this effect is very small.

The aqueous solutions of sugars have low dielectric constant [169] than pure water indicating that the electrostatic interactions should be stronger in these solutions than in pure water. However, this contribution to the stabilizing effect must be relatively small as compared to the hydrophobic interactions.

Hydrophobic interactions are generally considered to be the major single factor in stabilizing the three dimensional structure of proteins [170]. In aqueous-organic mixed solvents, hydrophobic interactions depend on the solvent structure,

Table 4.5: Intrinsic Viscosity, ([η],ml/g) as functions of temperature and pH of the following systems:

(a) ***Ovalbumin- Buffer System***

pH of the system	Temperature K 298.15	303.15	308.15	313.15	318.15
2.4	9.2737	10.5598	9.6547	9.7910	10.1529
7.0	3.0793	03.3879	3.7121	3.9709	04.0628
8.9	7.2204	05.7842	5.0946	9.7505	06.0049

(b) ***Ovalbumin-Maltose-Buffer System***

pH of the system	Temperature K 298.15	303.15	308.15	313.15	318.15
2.4	3.0625	3.2579	3.6162	3.8992	4.2352
7.0	3.1891	3.3079	3.3156	3.7311	4.1225
8.9	3.2854	3.4130	3.8160	4.1800	4.2634

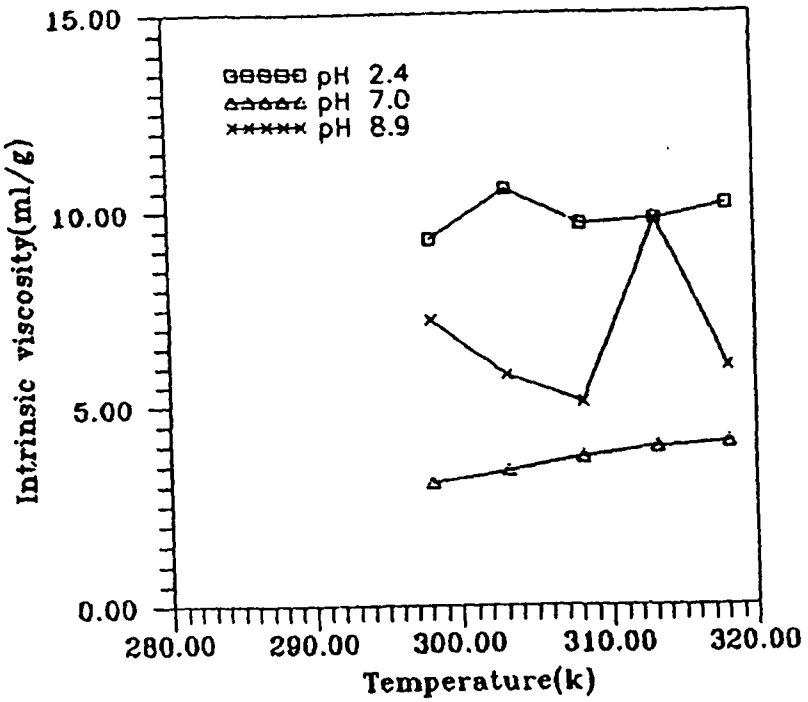

Fig 4.4(a) Plots of intrinsic viscosity versus temperature for ovalbumin at different pH values

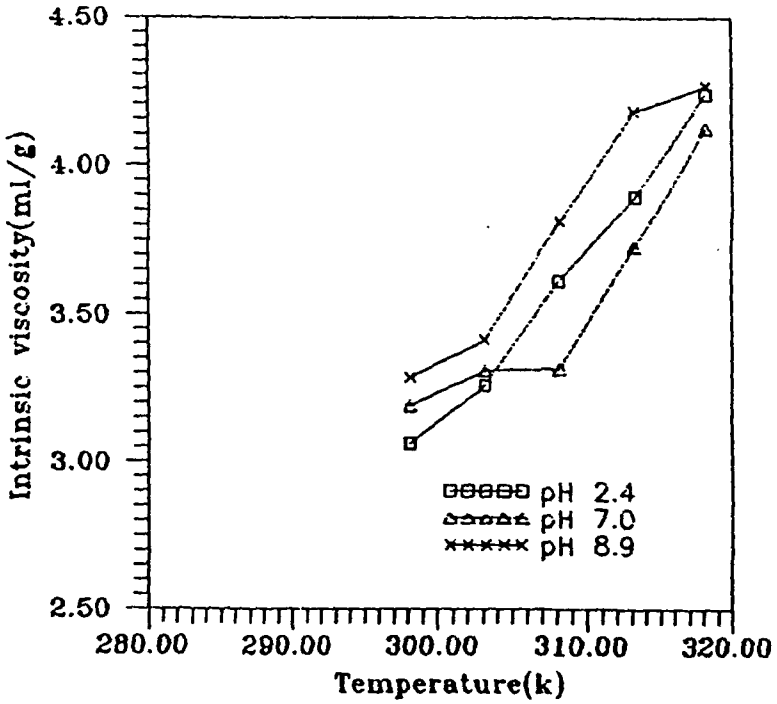

Fig 4.4(b) Plots of intrinsic viscosity versus temperature for ovalbumin–maltose system at different pH values

Table 4.6: Shape factor, ν, of ovalbumin as functions of temperature and pH of the following systems:

(a) ***Ovalbumin- Buffer System***

pH of the system	Temperature K 298.15	303.15	308.15	313.15	318.15
2.4	8.7465	9.8672	8.5079	8.6867	9.2467
7.0	3.0026	3.3594	3.7136	3.9363	3.9535
8.9	5.3658	5.0525	5.0143	9.9173	5.0924

(b) ***Ovalbumin-Maltose-Buffer system***

pH of the system	Temperature K 298.15	303.15	308.15	313.15	318.15
2.4	3.0256	3.0738	3.3869	3.9234	4.1079
7.0	3.0010	3.0491	3.0547	3.4327	3.6903
8.9	3.4418	3.4958	3.7932	4.1775	4.3802

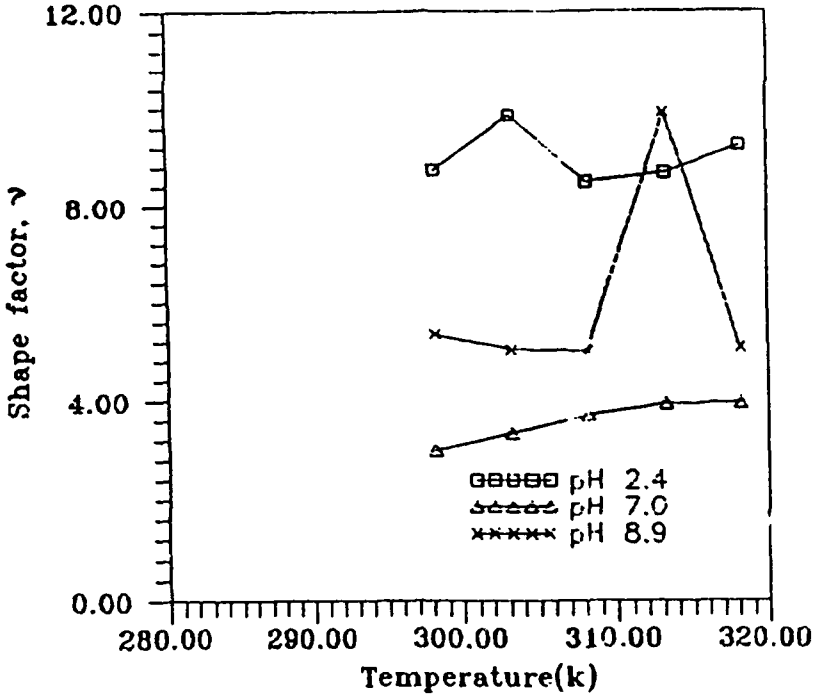

Fig 4.5(a) Plots of shape factor versus temperature for ovalbumin at different pH values

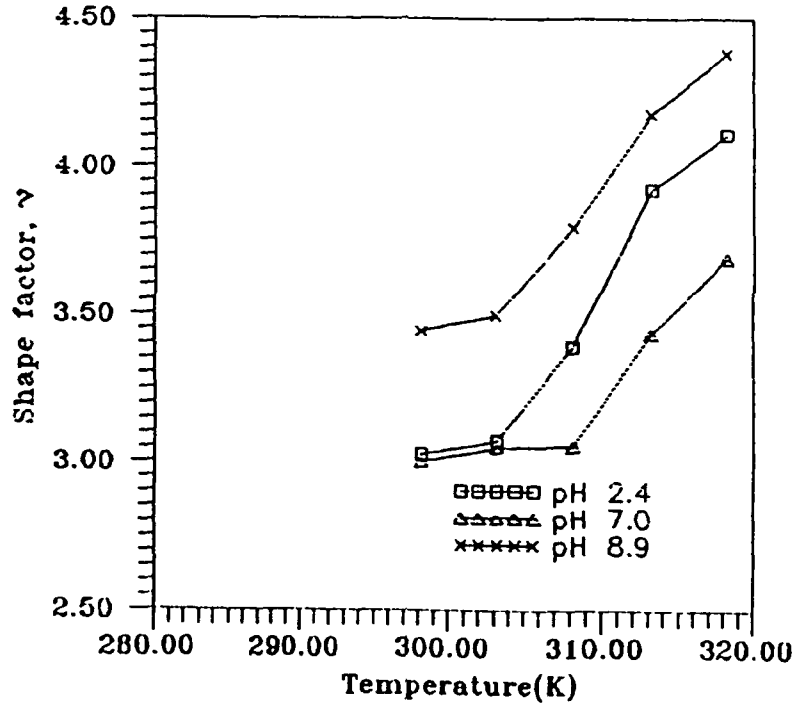

Fig 4.5(b) Plots of shape factor versus temperature for ovalbumin–maltose system at different pH values

with maximum hydrophobic interactions occurring in those solvent mixtures in which the three dimensional structure of water is most developed [171]. Evidence derived from both spectroscopy and thermodynamics shows that sugars interact with water to an extent, which depends upon their molecular structure [172]. Sugar molecules induce structure in the water molecules surrounding them [172]. The protective action of sugars on proteins can be attributed to the fact that sugars may replace a certain number of water molecules that are hydrogen bonded to the structure in a way similar to water itself creating a hydrophilic surface. This would result in a solvent system where the already exposed peptide attached with non-polar groups in the native protein molecule would have a tendency to enter into the protein interior due to unfavorable environment produced by sugar molecules. Similar groups in the interior of the protein would find an even more unfavorable environment in sugar solutions than in pure water on their exposure. This would result in more stability of a protein molecule in these solvents and would reduce the extent of denaturation of protein molecules induced thermally or by extremes of pH or other denaturing agents.

An examination of tables 4.5 a and b show that ovalbumin undergoes denaturation at the extremes of pH, hence the value of $[\eta]$ is very large. The addition of maltose sugar to the protein stabilizes it through increased hydrophobic interactions, therefore, in this case the trend of variation of $[\eta]$ is the same as that at pH 7.0. This indicates that the extent of stabilization is nearly the same from pH 2.4 to 8.9 and the stabilization is independent of pH.

The values of shape factor follow exactly the same pattern as that of $[\eta]$. Fig 4.5 (a) clearly indicates that the thermal unfolding of the protein occurs in steps while the stepwise stabilization of modified protein is not so pronounced (fig 4.5 b).

The viscosity coefficients for amino acid–urea-water systems have been expressed interms of Jones-Dole equation. Amino acids are dipolar ions in solutions. The viscosities of solutions of dipolar ions display non-electrolyte

behaviour, therefore, no A-coefficient is required to fit the data and equation 4.6 becomes.

$$\eta_r = (\eta'/\eta) = 1 + Bc + Dc^2 \qquad 4.7$$

$$\eta_r/c = (\eta_r - 1)/c = Bc + Dc^2$$

For each dipolar ion and the temperature a plot of $(\eta_r - 1)/c$ vs c was constructed and the B-coefficient was evaluated from the intercept and the D-coefficient from the slope. Figures 4.6 (a) to (d) show plots of the data obtained for amino acids L-valine and L-serine in urea-water mixtures at various temperatures. Results obtained for the B and D-coefficients are tabulated in table 4.8.

When a solute is dissolved in a solvent a hole is made in the liquid rupturing the intermolecular bonds and the solute is inserted. Some of the solvent molecules are attached with the ions because of ion-solvent interactions and this causes an increase in the viscosity of the solution. This contributes positively to the viscosity B-coefficient. On the other hand, the breaking of the solvent structure by solutes causes a decrease in the viscosity and thus contributing negatively to the B-coefficient. Thus, the B-coefficient is the resultant of these two opposite forces [19]. Therefore, the molecules/ions exhibiting negative B-coefficient have been assumed to exert a structure-breaking effect on the solvent, while ions with positive B-coefficients are termed structure-making. In the present case, L-valine belongs to the category of amino acids having hydrocarbon chain while L-serine belongs to the group containing a hydroxyl group attached to the hydrocarbon chain of the amino acid. But according to the tables 4.8, when the data was compared it was found that in an amino acid the charge distribution is less important than the size and structure of the hydrocarbon chain in determining the viscosities [119]. Due to their large size both the amino acids contain significant positive core contributions to the B-coefficient which exceeds any negative contribution due to a structure-breaking effect on the solvent. A large enough ion or molecule will necessarily exhibit a positive B-coefficient. Therefore, the sign of dB/dT appears to be a more straight-forward indicator of the structure-breaking or making ability than the sign or size of the B-coefficient [119]. A positive dB/dT

Table 4.7: η_{sp}/c (l mol^{-1}) as functions of temperature and concentration for the following systems:

(a) ***L-Valine-Urea-Water System***

Concentration mol/l	Temperature K 298.15	303.15	308.15	313.15	318.15
0.050	0.2604	0.3036	0.3313	0.3075	0.3607
0.100	0.2992	0.2968	0.3182	0.3364	0.2960
0.150	0.3952	0.3925	0.3855	0.3574	0.3547
0.200	0.3948	0.3960	0.3931	0.3795	0.3698
0.250	0.4091	0.4138	0.4091	0.3961	0.3814
0.300	0.4337	0.4314	0.4194	0.4005	0.3853
0.350	0.4506	0.4470	0.4310	0.4092	0.3954
0.400	0.4673	0.4617	0.4415	0.4167	0.4042

(b) ***L-Serine-Urea-Water System***

Concentration mol/l	Temperature K 298.15	303.15	308.15	313.15	318.15
0.100	0.0552	0.1380	0.1844	0.1716	0.1425
0.160	0.2290	0.2857	0.3159	0.2665	0.2657
0.240	0.2702	0.2948	0.3087	0.2965	0.2894
0.320	0.2801	0.2972	0.3075	0.2951	0.2899
0.426	0.2690	0.2810	0.2865	0.2807	0.2826
0.480	0.3014	0.3113	0.3157	0.3068	0.3019
0.560	0.3129	0.3174	0.3170	0.3065	0.2997
0.640	0.3068	0.3189	0.3244	0.3189	0.3094
0.720	0.3166	0.3232	0.3250	0.3185	0.3123
0.857	0.3001	0.3048	0.3060	0.3000	0.2952

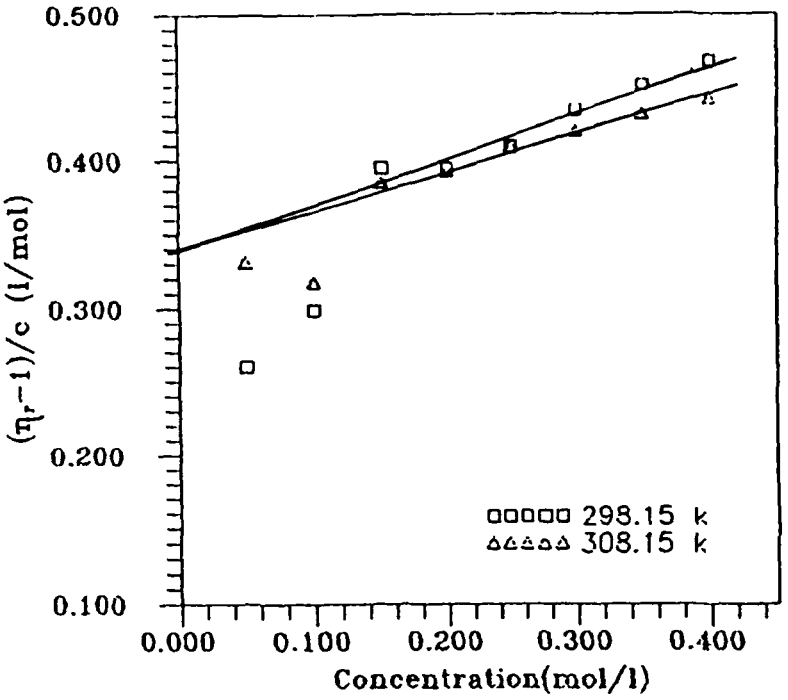

Fig 4.6(a) Plots of $(n_r-1)/c$ versus concentration for L-valine-urea-water system

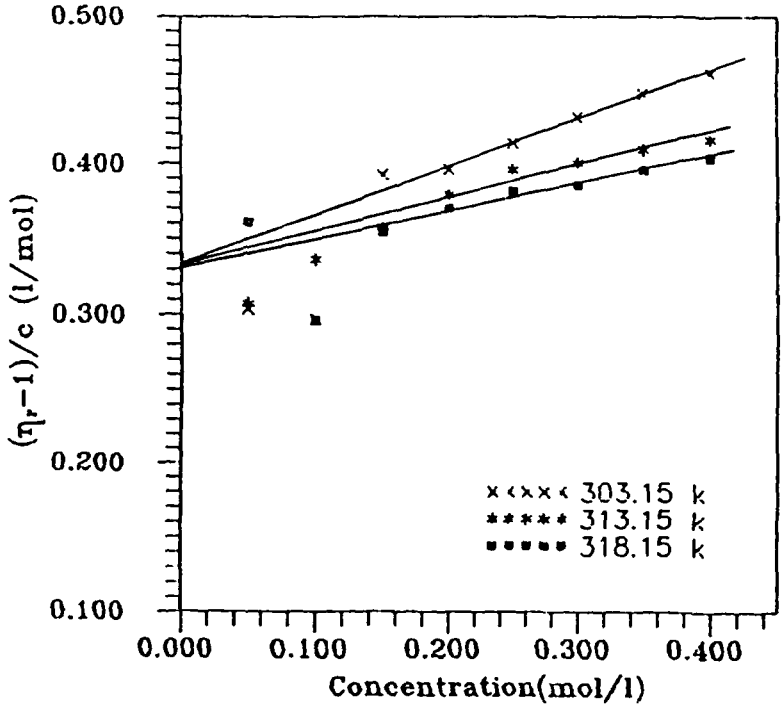

Fig 4.6(b) Plots of $(n_r-1)/c$ versus concentration for L-valine-urea-water-system

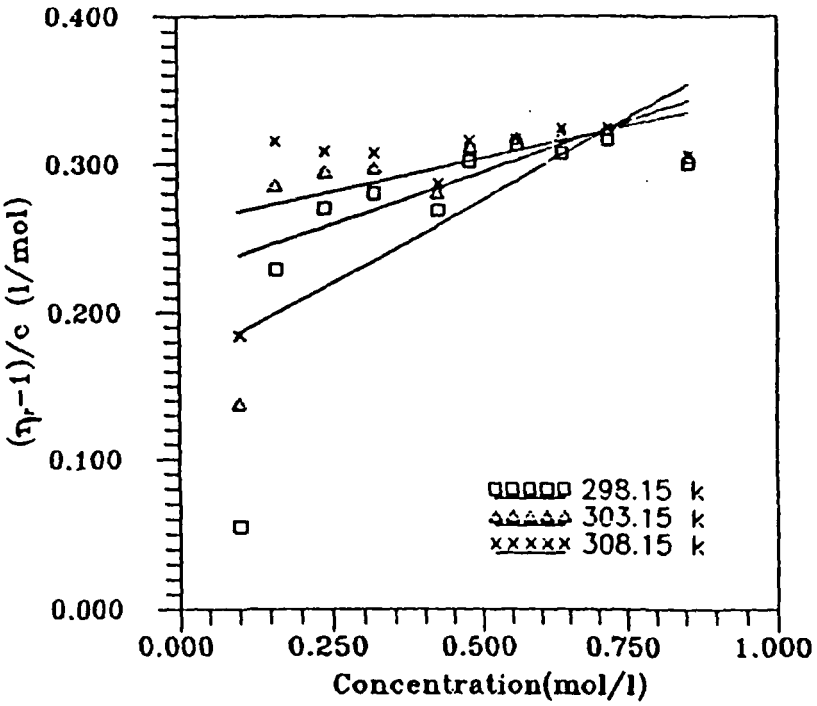

Fig 4.6(c) Plots of $(n_r-1)/c$ versus concentration for L-serine-urea-water system

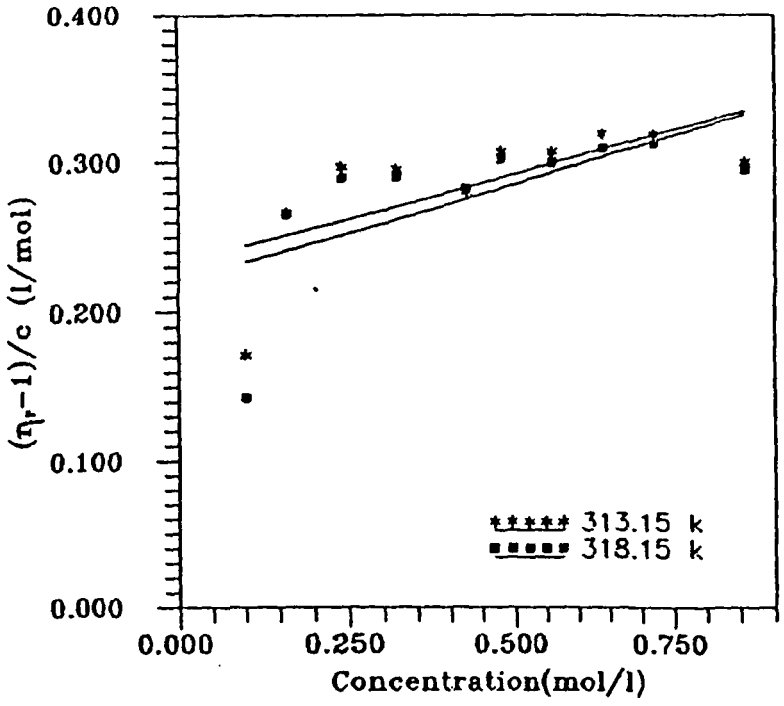

Fig 4.6(d) Plots of $(n_r-1)/c$ versus concentration for L-serine-urea-water system

indicates a structure-breaking ion or molecule and a negative sign, a structure-making one. In this case, the B-value for serine increases with the increase in temperature upto 35° C. Since dB/dT is positive, we can classify it as a structure-breaker in urea-water mixture. Its structure-breaking property is due to the presence of a polar R group which is involved in the hydrogen bonding with the solvent molecules resulting in the breaking of solvent components. We observe a decrease in the value of B-coefficient after 35° C, i.e., a negative dB/dT at 40° and 45 °C. This might be a result of decrease in solvation at higher temperatures. As evident from fig 4.7, the B-coefficient for valine decreases with increase in temperature. Since dB/dT is negative, we can say that valine is structure-maker. Its structure-making property is due to the presence of hydrophobic (apolar) R group which stabilizes the structure of the solvent through hydrophobic hydration with the solvent components. In this case the probability of hydrogen bonding between the molecules of the solvents is increased by forming solvent clathrate around the hydrophobic moiety.

Table 4.8: B and D coefficients of equation 4.7 for relative viscosities of the dipolar ions in urea-water mixture:

	Temperature K				
	298.15	303.15	308.15	313.15	318.15
L-Valine					
$(molar^{-1})B$	0.3390	0.3325	0.3130	0.3320	0.3310
$(molar^{-2})D$	0.0556	0.0474	0.0346	0.0303	0.0254
L-Serine					
$(molar^{-1})B$	0.1643	0.2250	0.2590	0.2326	0.2191
$(molar^{-2})D$	0.2217	0.1383	0.0891	0.1188	0.1317

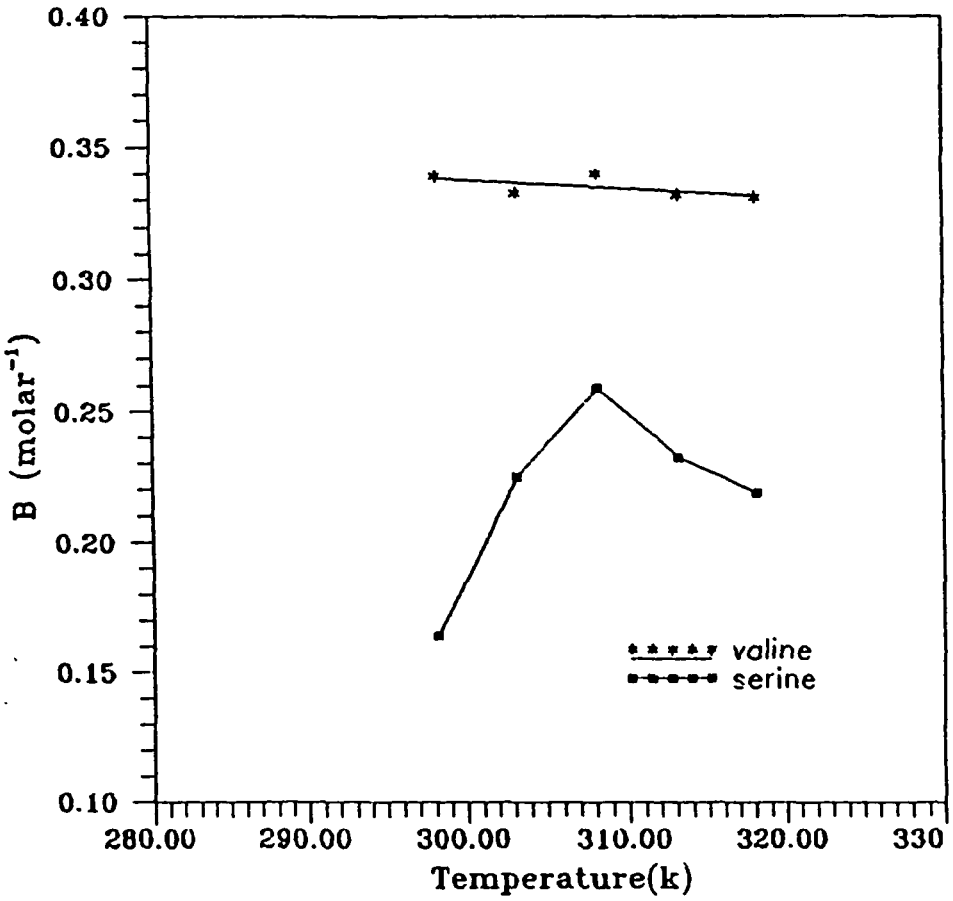

Fig 4.7 Plots of viscosity B coefficient versus temperature for amino acid–urea–water system

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